BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problems Mailbox.

STIC-ILL 337,255

From:
Sent: Saturday, March 17, 2001 5:45 PM
To:
Subject:

Please get the following journal articles and have them delivered to my mailbox in CM1-3B19:

Aoki, S.; Okamoto, A.; Danjo, K.; Sunada, H.; Otuka, A. 'Compatibility of ibuprofen and ethenzamide' Drug Dev. Ind. Pharm. (1997) 23(6):561-565.

Murtha, John L.; Ando, HOward Y. 'Synthesis of hte cholesteryl ester prodrugs chloesteryl ibuprofen and cholesteryl flufenamate and their formulation into phospholipid microemulsions' J. Pharm. Sci. (1994) 83(9):1222-8.

Goroshevich, R. V.; Kosmynin, A. S.; Rozhanskaya, A. E.; Tkachenko, M. L. 'Physicochemical study of drug binary systems' Khim.-Farm. Zh. (1992) 26(2):73-6.

Mura, P.; Liguori, A.; Bramanti, G. 'Solid dispersions of ibuprofen in urea. Effects of urea on dissolution' Farmaco, Ed. Prat. (1986) 41(12):377-87.

Rogers, J. A.; Anderson, A. J. 'Physical characteristics and dissolution profiles of ketoprofen-urea solid dispersions' Pharm. Acta Helv. (1982) 57(10-11):276-81.

ND 3/19

LC
3/20
SMP
Completed
0
5
,50

Индекс 71481

предприятий и
изаций (ЦАЗИ)
3 р. 50 к.

ISSN 0023-1134

Химико-фармацевтический журнал

МОСКВА · МЕДИЦИНА · 1992

2

ISSN 0023-1134. Хим.-фарм. журнал. 1992. Т. 26. № 1. 1—88.

(*а*) аминировать до II и, наконец, II формилировать до I. Оба способа (*а*) и (*б*) получения I являются многостадийными.

Нами разработан непрерывный одностадийный способ получения I, основанный на прямом взаимодействии IV с водным раствором формальдегида (V) в присутствии анионообменной смолы АВ-17-8 в ОН-форме и аммиака, который служит как реактивирующий агент для катализатора.

Экспериментальная часть

Синтез I проводили в термостатированном реакторе проточного типа с внутренним диаметром 12 мм и высотой 300 мм. Через стационарный слой анионита АВ-17-8 (30 мл) при температуре 60—90 °С нисходящим потоком непрерывно пропускали раствор, содержащий IV (9,58 %) и V (5,11 %) в воде, добавляли 0,5—1,5 моля аммиака. Длительность реакции 1 ч. Срок службы катализатора в непрерывном процессе 40 ч.

Получено 16,6 г (0,109 моля) I, что соответствует 75,7 % от теории в расчете на IV; получено также 4,25 г (0,0319 моля) II, что составляет 23,2 % на IV. При атмосферном или пониженном давлении отгоняли жидкую часть реакционной смеси и снова возвращали в реакцию. Сухой остаток перекристаллизовывали из спирта. Полученный I представляет собой белые кристаллы с т. пл. 147—149 °С $C_7H_8N_2O_2$. Данные элементного анализа удовлетворяют вычисленным значениям. Спиртовую вытяжку дополнительно отгоняли и получали II с т. пл. 130—131 °С. $C_6H_5N_2O$.

Ик-спектры снимали на спектрометре UR-20 в таблетках с KBr в соотношении 1:200 в области 400—4000 $см^{-1}$. Наиболее интенсивные характеристические полосы поглощения в ИК-спектре I: (ν, $см^{-1}$): 1030 (С—О), 1320 (С—N), 1610 (скелетн. колебания цикла), 1690 и 1705 (дублет, С=О); 2840 и 2940 (CH_2); 3070 (С—Н), 3000—3100 (ОН), 3340 (N—Н).

SUMMARY

The paper outlines a continuous heterogeneous catalytic procedure to prepare nicotinic acid hydroxymethylamide from 3-cyanopyridine in a flow reactor.

ЛИТЕРАТУРА

1. *Машковский М. Д.* Лекарственные средства.— М., 1977.— Ч. 1.— С. 458.
2. *Жданович Е. С., Чекмарева И. Б.* и др. // Мед. пром-сть СССР.— 1962.— Т. 3.— С. 25—26.
3. *Чекмарева И. Б., Янина М. М., Жданович Е. С.* и др. // Ионный обмен и иониты / Под ред. Г. В. Самсонова.— Л., 1970.— С. 322.
4. *Никифоров В. А., Герасимова Л. Е., Строганова И. И., Гусейнов Э. М.* и др. // Хим.-фарм. журн.— 1973.— № 8.— С. 43—46.
5. *Никифоров В. А., Григорьев Т. А., Строганова И. И.* и др. // Там же.— 1976.— Т. 10, № 9.— С. 127—129.
6. *Гусейнов Э. М., Строганова И. И., Беликова Н. В.* // Там же.— № 11.— С. 114—116.

Поступила 21.06.91

УДК 615.7:543.226:532.73

Р. В. Горошевич, А. С. Космынин, А. Э. Рожанская, М. Л. Ткаченко

ФИЗИКО-ХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ БИНАРНЫХ СИСТЕМ ЛЕКАРСТВЕННЫХ ВЕЩЕСТВ

Куйбышевский медицинский институт им. Д. И. Ульянова

Определяющим моментом биодоступности трудно и ограниченно растворимых препаратов для внутреннего применения является скорость перехода в раствор активного начала, поскольку всасывание происходит только в растворенном состоянии [5]. Увеличение скорости растворения путем механической микронизации веществ не всегда достижимо. Это объясняется наличием процессов агломерации и агрегации кристаллов, ухудшением смачиваемости сверхтонкого порошка.

В настоящих исследованиях отражены результаты поиска оптимальной дисперсности, а также оценка взаимовлияния компонентов систем на их растворение, выполненная приемами и методами физико-химического анализа.

Исследования проводились на модельных бинарных системах биологически активных веществ. В качестве исходных компонентов использовали субстанции препаратов амидопирина, фенацетина, анестезина, никотинамида, левомицетина и мочевины, отвечающие требованиям нормативно-технической документации. Вещества подвергали дополнительной очистке многократной перекристаллизацией из спирто-водных растворов до постоянной температуры плавления. Идентификацию и оценку степени чистоты осуществляли методами рентгенофазового (РФА), дифференциального термического (ДТА) и спектрофотометрического анализа.

РФА проводили на установке ДРОН-3,0 с CuK_α излучением, кварцевым монохроматором, внутренним стандартом из металлического германия полупроводниковой чистоты. Расшифровка рентгенограмм осуществлялась с привлечением картотеки ASTM. Все пики на рентгенограммах фенацетина, анестезина, левомицетина, мочевины идентифицированы по основному веществу. Для образцов амидопирина и никотинамида отмечено несколько неидентифицируемых пиков с интенсивностью менее 5 %, что указывает на наличие микропримесей, которые не устраняются использованными методами очистки.

Температуры плавления и термическую устойчивость препаратов определяли методом ДТА на установке, состоящей из печи нагрева шахтного типа, программатора нагрева — охлаждения, усилителя ЭДС дифференциальной термопары и двухточечного автоматического потенциометра типа КСП. Использовали тигли из алюминия, ско-

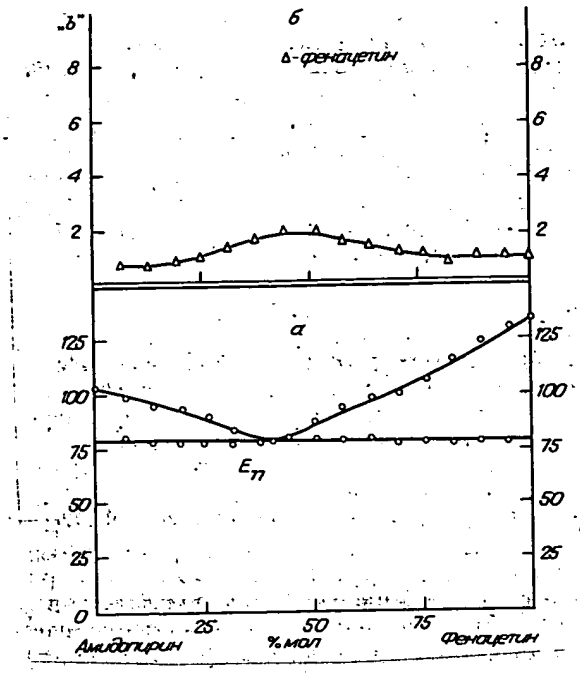


Рис. 1. Диаграммы плавкости (*а*) и состав — скорость растворения (*б*) системы амидопирин — фенацетин.
Здесь и на рис. 2—3 по оси абсцисс — состав, % мол.; по оси ординат — температура, °С.

рость нагрева — охлаждения 10 °С/мин, масса образцов около 0,1 г.

Термическую устойчивость контролировали по температурам плавления веществ в режиме 3-кратных циклов нагрев — охлаждение. Исследования показали, что температуры плавления амидопирина, фенацетина, анестезина, никотинамида, левомицетина, мочевины стабильны и соответственно равны 107, 135, 90, 130, 149, 128 °С. Отмеченные температуры в пределах погрешности экспериментов не противоречат данным литературы [3, 7].

Отсутствие продуктов разложения после плавления параллельно доказано методом РФА.

Из образцов сформированы три бинарные системы в сочетаниях амидопирин — фенацетин, анестезин — никотинамид, левомицетин — мочевина с варьированием соотношений компонентов через 5 %. Геометрическая интерпретация данных термических экспериментов позволила определить тип физико-химического состояния систем (рис. 1—3). Все три системы относятся к эвтектическому типу, составы и температуры плавления эвтектик приведены в табл. 1. Ликвидусы фазовых диаграмм дополнительно уточнены методом визуально-политермического анализа (ВПА). Рентгенофазовые исследования образцов сплавов во всем интервале систем показали отсутствие в них новых фаз, что подтверждает данный тип диаграмм состояния.

Для всех составов вышеперечисленных систем определяли скорость растворения компонентов из механических смесей и сплавов в раствор хлористоводородной кислоты с pH 1,2 в объеме 500 мл. Кинетические эксперименты проводили на приборе с вращающейся мешалкой, перемешивание среды осуществляли со скоростью 100 об/мин. Исследуемые составы таблетировали прямым прессованием под давлением 5 МПа, масса таблеток — 0,3 г, диаметр — 12 мм. Для формования отбирали фракции дисперсностью 200 мкм. Плавленые образцы охлаждали в стеклянной ступке, погруженной в лед. Таблетки фиксировали на дне термостатированного сосуда. Пробы отбирали с периодичностью 2, 4, 6, 8, 10, 15, 20, 25, 30 мин, объемы их компенсировали чистым растворителем. Количественное определение ингредиентов осуществляли спектрофотометрически. Концентрации рассчитывали методом Фирордта (за исключением системы левомицетин — мочевина) по следующим уравнениям [4, 6]:

Рис. 2. Диаграммы плавки (*а*) и состав — скорость растворения (*б*) системы анестезин — никотинамид.

$$C_1 = \frac{E_2^{\lambda 2} \cdot D^{\lambda 1} - E_2^{\lambda 1} \cdot D^{\lambda 2}}{(E_1^{\lambda 1} \cdot E_2^{\lambda 2} - E_1^{\lambda 2} \cdot E_2^{\lambda 1}) \cdot l},$$

$$C_2 = \frac{E_1^{\lambda 1} \cdot D^{\lambda 2} - E_1^{\lambda 2} \cdot D^{\lambda 1}}{E_1^{\lambda 1} \cdot E_2^{\lambda 2} - E_1^{\lambda 2} \cdot E_2^{\lambda 1}) \cdot l},$$

где $E_1^{\lambda 1}$, $E_1^{\lambda 2}$ и $E_2^{\lambda 1}$, $E_2^{\lambda 2}$ — удельные показатели поглощения компонентов C_1 и C_2 при длинах волн λ_1 и λ_2, $D^{\lambda 1}$ и $D^{\lambda 2}$ — оптические плотности при длинах волн λ_1 и λ_2, l — толщина кюветы.

Для системы левомицетин — мочевина определяли лишь концентрацию антибиотика, так как мочевина в исследуемом диапазоне длин волн не имеет максимумов светопоглощения.

Предварительно была осуществлена проверка воспроизводимости методики в шести повторностях для системы амидопирин — фенацетин. Относительная ошибка измерений с учетом 95 % доверительного интервала составила ±1,37 %.

По результатам определения степени перехода в раствор компонентов из образцов построены кинетические кривые для всех составов систем. Профили кривых выхода компонентов из каждой бинарной системы имели одну форму. Причем

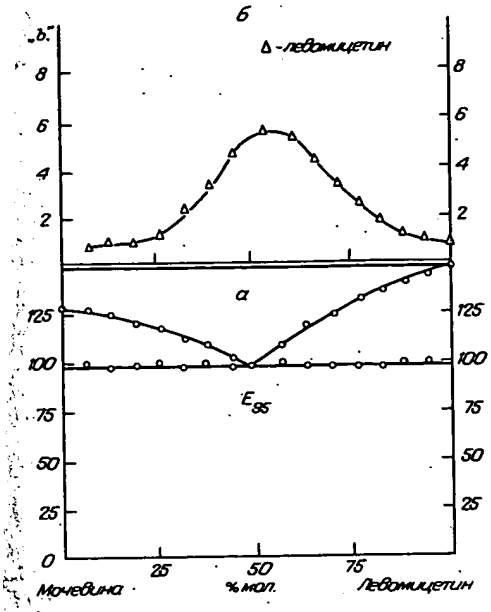


Рис. 3. Диаграммы плавкости (*а*) и состав — скорость растворения (*б*) системы левомицетин — мочевина.

аналогичными были профили растворения как чистых субстанций, так и соответствующих образцов плавов и механических смесей.

Способом приведения к линейному виду методом наименьших квадратов участка кривых до выхода на плато определена их интенсивность наклона, количественно охарактеризованная угловым коэффициентом «*b*» уравнения прямой: $y = bx + a$, где y — концентрация перешедшего в раствор вещества (в мкг/мл), x — время (в мин). Доказательством линейной зависимости и показателем жесткости линейной связи между значениями концентраций компонентов в растворе и временем растворения является величина коэффициента корреляции «*r*», которая во всех случаях превышала значение 0,95.

По экспериментальным данным для исследуемых систем построены диаграммы состав — скорость растворения (см. рис. 1—3), которые наглядно отображают взаимное влияние компонентов на кинетику растворения. При совместном анализе диаграмм плавкости и скорости растворения выявлено, что именно эвтектические составы характеризуются максимальным увеличением скорости перехода в раствор формирующих систему компонентов по сравнению с аналогичными параметрами исходных субстанций.

Эффекты увеличения скоростей выхода плохо растворимых компонентов из эвтектических составов приведены в табл. 2.

Наблюдаемые эффекты при отсутствии взаимодействия компонентов смесей, по-видимому, объясняются уменьшением степени кристалличности и солюбилизирующим влиянием веществ. Как известно, при охлаждении компонентов эвтектической смеси происходит их одновременная кристаллизация с максимальной степенью дисперсности твердых фаз. В свою очередь возрастание удельной поверхности служит одним из факторов повышения скорости растворения. Немаловажную роль играет фактор отсутствия или значительного уменьшения агрегации и агломерации частиц эвтектических составов. Величина эффектов роста скорости зависит также от разности растворимости исходных компонентов систем. Влияние солюбилизирующего действия количественно можно охарактеризовать индексами, определяемыми как отношение величин угловых коэффициентов исходных компонентов смесей. Для модели амидопирин — фенацетин индекс равен 30, для системы анестезин — никотинамид — 10. Исследованиями продемонстрирован наибольший эффект увеличения скорости растворения для пары левомицетин — мочевина (см. табл. 2) с использованием гидрофильного носителя, способствующего смачиванию поверхности кристаллов.

Тождественность диаграмм состав — скорость растворения диаграммам плавкости подтверждает также результаты исследований ДТА, ВПА, РФА об отсутствии взаимодействия компонентов систем. Еще одним аргументом в пользу достоверности полученных данных является идентичность УФ-спектров и удельных показателей поглощения растворов как физических смесей, так и таблетированных их образцов, а также плавов ряда составов систем.

Таким образом, результаты исследований показали целесообразность применения методов ДТА, ВПА, РФА для описания свойств систем в целом как достаточно точных способов быстрого выяснения совместимости ингредиентов в комбинированных лекарственных формах, их стабильности при «температурных стрессах», что отразило преимущество системного анализа перед препаративными химическими методами.

Описанные комплексные исследования систем приемами и методами физико-химического анализа однозначно исключили вероятность развития эффектов объемного взаимодействия при таблети-

Таблица 1

Составы и температуры плавления эвтектик систем

Система	Состав эвтектики, % мол. (% мас.)	Температура, °С
Амидопирин — фенацетин	65 (71) 35 (29)	77
Анестезин — никотинамид	33 (40) 67 (60)	73
Левомицетин — мочевина	50 (84) 50 (16)	95

Таблица 2

Скорость растворения труднорастворимого компонента из эвтектических смеси и плава систем

Система	Труднорастворимый компонент	Скорость растворения индивидуального вещества, «b»	Скорость растворения из эвтектического состава, «b»		Прирост скорости растворения	
			смеси	плава	смеси	плава
Амидопирин — фенацетин	Фенацетин	1,1	1,3	1,7	1,2	1,5
Анестезин — никотинамид	Анестезин	8,2	8,5	9,0	1,0	1,1
Левомицетин — мочевина	Левомицетин	1,2	4,3	5,5	3,3	5,0

ровании в жесткой пресс-форме, непредвиденных изменений в составе препаратов за счет выделения теплоты в процессе прессования [1].

Установлена возможность применения плавления в качестве способа подготовки исследуемых лекарственных смесей к таблетированию путем получения их тонкодисперсных смесей. Сплавы эвтектического состава обеспечивают наряду с этим стабилизацию дисперсного состояния фаз, гомогенность смеси компонентов и повышение точности дозирования в результате более эффективного перемешивания ингредиентов в расплавленном состоянии.

Данные экспериментов могут представлять практический интерес и для рационального технологического решения проблемы [2] безопасного измельчения склонных к воспламенению в режиме механического диспергирования лекарственных веществ (анестезин, левомицетин).

Исследования имели целью продемонстрировать полиинформативность использования физико-химического анализа в решении ряда биофармацевтических проблем конструирования многокомпонентных твердых лекарственных форм. Этапы работы могут представлять программу поиска композиций медицинского назначения с заданными свойствами на основе анализа диаграмм состояния многокомпонентных систем с доказательством согласованного изменения свойств равновесных систем (в данном случае растворимости) в зависимости от их состава.

SUMMARY

Physicochemical analytical methods were used to examine the following model system: amidopyrine-phenacetin, anesthesine-nicotinamide, levomycetin-urea. All the three systems were ascertained to be of eutectic type. The interaction of binary system components was analyzed on the kinetic of their transition to hydrochloric acid solution having pH 1.2 from mechanical mixtures and fusion cakes. The liquescency diagram and the solubility diagram were constructed for the systems. The comparison of the diagrams revealed their relationship. The transition rate for components to solution was found to be the maximum for eutectic compositions. The experimental findings may be applied to predict the release rate for components of solid drug compositions, to modify their composition, to implement their process more rationally.

ЛИТЕРАТУРА

1. *Белоусов В. А.* // Хим-фарм. журн.— 1987.— № 11.— С. 1355—1361.
2. *Бондарь В. А., Марасанова В. А., Попов Б. Г.* и др. // Там же.— 1990.— № 3.— С. 70—72.
3. Государственная фармакопея СССР.— 10-е изд.— М., 1968.
4. *Гулимова Т. Е.* // Всероссийский съезд фармацевтов, 4-й: Тезисы докладов.— Воронеж, 1981.— С. 318—319.
5. *Михайлова Г. С.* // Фармация.— 1974.— № 1.— С. 80—83.
6. *Пиняжко Р. М., Каленюк Т. Г.* Методы УФ-спектрофотометрии в фармацевтическом анализе.— Киев, 1976.
7. *Сарян Л. А., Попков В. А., Дугачева Г. М.* // Фармация.— 1989.— № 1.— С. 23—25.

Поступила 27.11.90

УДК 615.2/.3.011.47:579.8].014.453

Е. П. Павлов, Э. Г. Тушов, И. И. Самойленко, Г. Я. Кивман, В. В. Дегтяренко, Н. С. Николаенко, М. И. Эльнатанова, Т. А. Шуб, Е. В. Бухарцева

ИСПОЛЬЗОВАНИЕ ГАММА-ИЗЛУЧЕНИЯ ДЛЯ МИКРОБНОЙ ДЕКОНТАМИНАЦИИ ЛЕКАРСТВЕННЫХ СРЕДСТВ

Институт биофизики Минздрава СССР

Проведенные в стране и за рубежом исследования показали, что одним из перспективных методов снижения микробной загрязненности лекарственных средств, выпускаемых в нестерильном виде, является облучение лекарств, лекарственного сырья и вспомогательных веществ небольшими дозами ионизирующих излучений [1, 2, 5—8]. Помимо высокой эффективности радиационного метода, практически ценно то, что его использование допускает обработку продукции в транспортной упаковке, а сам процесс обработки технологичен и может быть полностью механизирован и автоматизирован.

В данной работе представлены результаты изучения эффективности снижения гамма-излучением микробной загрязненности лекарств, выпускаемых Нижегородским химико-фармацевтическим заводом.

Экспериментальная часть

Качественный и количественный состав лекарственных средств (свечи, мази, линименты, суппозитории, шарики, гель, масло) до облучения и после облучения гамма-излучением в дозах 1 и 5 кГр определяли в соответствии с рекомендациями, изложенными в Государственной фармакопее XI издания в разделе «Испытание на микробиологическую чистоту» (вып. 2, с. 193—209). В других опытах изучали эффективность облучения в дозах 1 и 5 кГр лекарственных препаратов, экспериментально контаминированных смешанной производственной микрофлорой, которая была представлена сапрофитными радиочувствительными и радиоустойчивыми микроорганизмами (золотистым стафилококком, кишечной палочкой, синегнойной палочкой и кандидами). Величины D_{10} (дозы излучения, снижающие численность микроорганизмов в 10 раз) для этих микроорганизмов составляли 0,1—2,0 кГр. Моделировали 2 производственные ситуации: 1) относительно небольшая микробная загрязненность лекарств — порядка 10^4 микроорганизмов в 1 г (мл) вещества; 2) массивная загрязненность лекарств — до уровня 10^5—10^6 микроорганизмов в 1 г (мл) вещества. Использовали препараты, неактивные в отношении данных тест-микроорганизмов: свечи с гидрокарбонатом натрия, мазь метилурациловую, мазь гидрокортизоновую и свечи с глицерином. Вещества смешивали с микроорганизмами, суспендированными в 1 % растворе твина-80. В опытах с препаратами, контаминированными небольшим коли-

STIC-ILL

GAU
RSI. 158

From: Berman, Alysia
Sent: Saturday, March 17, 2001 6:45 PM
To: STIC-ILL
Subject: 09/423715

Please get the following journal articles and have them delivered to my mailbox in CM1-3B19:

Aoki, S.; Okamoto, A.; Danjo, K.; Sunada, H.; Otuka, A. 'Compatibility of ibuprofen and ethenzamide' Drug Dev. Ind. Pharm. (1997) 23(6):561-565.

Murtha, John L.; Ando, HOward Y. 'Synthesis of hte cholesteryl ester prodrugs chloesteryl ibuprofen and cholesteryl flufenamate and their formulation into phospholipid microemulsions' J. Pharm. Sci. (1994) 83(9):1222-8.

Goroshevich, R. V.; Kosmynin, A. S.; Rozhanskaya, A. E.; Tkachenko, M. L. 'Physicochemical study of drug binary systems' Khim.-Farm. Zh. (1992) 26(2):73-6.

Mura, P.; Liguori, A.; Bramanti, G. 'Solid dispersions of ibuprofen in urea. Effects of urea on dissolution' Farmaco, Ed. Prat. (1986) 41(12):377-87.

Rogers, J. A.; Anderson, A. J. 'Physical characteristics and dissolution profiles of ketoprofen-urea solid dispersions' Pharm. Acta Helv. (1982) 57(10-11):276-81.

Thank you.

Alysia Berman
Patent Examiner
CM1-3D06
(703) 308-4638
1619

Synthesis of the Cholesteryl Ester Prodrugs Cholesteryl Ibuprofen and Cholesteryl Flufenamate and Their Formulation into Phospholipid Microemulsions

JOHN L. MURTHA[x] AND HOWARD Y. ANDO

Received May 10, 1993, from the *Philadelphia College of Pharmacy and Science, Department of Pharmaceutics, 600 S. 43rd Street, Philadelphia, PA 19104.* Accepted for publication February 28, 1994[a]. [x]Current address: Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company, Department of Pharmaceutics, 2800 Plymouth Road, Ann Arbor, MI 48105.

Abstract □ Phospholipid microemulsions have been suggested as a drug-delivery system for hydrophobic compounds. In this study hydrophobicity was achieved by derivatizing with cholesterol. Cholesteryl ibuprofen (3) and cholesteryl flufenamate (4) were synthesized. 3 was isolated as an amorphous, white solid with a melting range of 114–120 °C. 4 was isolated as a crystalline, white solid with a melting range of 145–148 °C. The proposed structures of 3 and 4 were supported by IR, NMR, MS, and organic microanalysis. Phospholipid:cholesteryl ester microemulsions were prepared by the addition of a 1-propanol solution of the cholesteryl ester, other lipids, and phospholipid to a rapidly mixing KCl/KBr solution. The hydrophobic phase was modified by the addition of cholesteryl oleate or triolein to study the effect of the fluidity of the hydrophobic core on the formation of the microemulsions. The results indicated that a molar ratio of 75:25 and a total lipid concentration of 60 mg/mL consistently gave microemulsions with a mean size of 100–150 nm. In addition, the formation of eutectic mixtures of 3 and 4 with cholesteryl oleate were determined to be 16% (w/w) for 3 and 12% (w/w) for 4; melting points were 35.2 and 45.2 °C, respectively. The solubilities of 3 and 4 in triolein were determined to be 13.2% (w/w) and 11.5% (w/w), respectively. Other investigators have shown that if the core of a phospholipid:cholesteryl ester microemulsion exists in a liquid state at physiologic temperature, the turnover of the cholesteryl esters from these microemulsions occurs at a faster rate. Future studies will focus on the turnover of cholesteryl ester prodrug fluidized cores on the bioavailability of the free drug in vivo.

I. Introduction

Lipoproteins and microemulsions have distinct advantages for use as drug-delivery systems over liposomes or vesicles.[1] They have in plasma a higher physical stability than vesicles, their hydrophobic internal phase is resistant to leaching, and hydrophobic drugs can be solubilized in the internal phase. In this study, two acidic drugs, ibuprofen and flufenamic acid, were made hydrophobic by forming their cholesteryl ester derivatives.

Lipid microemulsions have been defined to have a droplet size ranging from 10–250 nm[1-4] and exist in a state between a micellar dispersion and a microemulsion.[1] Several methods have been described for the preparation of lipid microemulsions; ultrasonication[5-7] is the most common. Although this method is capable of producing 20–100-nm microemulsions, long sonication times (1–12 h) can potentially lead to lipid and drug degradation. For this study, a milder technique, which has also been used to prepare liposomes, has been chosen. Cholesteryl ester and phospholipid are dissolved in 1-propanol and then added to the aqueous phase with vigorous stirring.[8,9] Formulation variables that could have an effect on microemulsion particle size and bioavailability of the cholesteryl ester prodrugs was also investigated.

II. Experimental Section

Reagents—Most reagents were used as received from the supplier: acetonitrile (HPLC grade), chloroform, cholesterol, hexane, methanol (HPLC grade), potassium chloride, potassium bromide, 1-propanol, 2-propanol, sodium phosphate dibasic, and tetrahydrofuran (HPLC grade) (Fisher Scientific Co., Pittsburgh, PA); cholesteryl oleate, L-α-dipalmitoylphosphatidylcholine, cholesteryl ester hydrolase (from porcine pancreas), cholesterol oxidase, flufenamic acid, horseradish peroxidase, *p*-hydroxyphenylacetic acid, 4-pyrrolidinopyridine, and triolein (Sigma Chemical Co., St. Louid, MO); 1,3-dicyclohexylcarbodiimide, polyethylene glycol 8000, and sodium taurocholate (Aldrich Chemical Co., Milwaukee, WI); ibuprofen (Mallinckrodt, St. Louis, MO; as a racemic mixture of 43% *R* and 53% *S* as determined by HPLC using the method of Berthod et al.[10]); ethyl acetate (EM Science, Cherry Hill, NJ); and 0.9% sodium chloride for injection USP (McGaw Inc., Irvine, CA). Anhydrous methylene chloride (Fisher Scientific, Pittsburgh, PA) was prepared by distillation over calcium hydride (Matheson Coleman & Bell, East Ruthr ford, NJ) and used immediately.[11]

Column Chromatography—The cholesteryl ester prodrugs were purified using column chromatography.[12,13] Silica gel (0–63 μm, Universal Scientific, Atlanta, GA) columns (2.5 cm diameter × 7 cm length) were prepared and equilibrated with hexane. A hexane solution of the reaction products was applied to the column and eluted with hexane. Approximately 10-mL fractions were collected using a fraction collector (FOXY 200 X-Y fraction collector, Isco, Inc., Lincoln, NE). The column effluent was monitored with a UV detector (SpectroMonitor III, Laboratory Data Control, Div. of Milton Roy Co., River Beach, FL) at 254 nm with the detector output recorded on a chart recorder (Perkin-Elmer Corp., Norwalk, CT).

Thin-Layer Chromatography—The synthetic reaction was monitored by TLC on silica gel plates (Whatman, silica gel, 60 Å, 1 × 3 in. 250-μm layer). Plates were developed using 80:20 hexane:ethyl acetate (R_f values: DCC = 0.02, cholesterol = 0.21, 1 = 0.56, 2 = 0.28, 3 = 0.77, 4 = 0.78).

Eluent fractions from column chromatography were monitored with TLC on silica gel plates (Fisher Scientific, Silica Gel GF, 20 cm × 20 cm, 250-μm layer). Plates were scored to form 20 1-cm-wide channels. Plates were developed using 80:20 hexane:ethyl acetate (R_f values: 3 = 0.75, 4 = 0.76) to verify only one compound was present in each fraction.

Melting Points—Two methods were used to determine melting ranges: a capillary melting point apparatus (A. H. Thomas Co., Philadelphia, PA) and a differential scanning calorimeter (DSC) (TA 3000 System with TC10 TA processor and DSC20 standard cell, Mettler Instrument Corp., Hightstown, NJ). Samples of 10–15 mg were weighed into vented DSC pans and scans run from 25 to 200 °C at 5 °C/min.

Infrared Spectroscopy—Infrared spectra of the reaction products were obtained on a FT-IR spectrometer (Model 1600, Perkin-Elmer Corp., Norwalk, CT) with a diffuse reflection accessory. Samples were triturated with KBr (IR Grade, Fisher Scientific, Pittsburgh, PA) and spectra obtained with a 1-min run time (16 scans).

Nuclear Magnetic Resonance Spectroscopy—NMR analysis of the reaction products (10% w/v in chloroform-*d*, 1% v/v TMS) was

[a] Abstract published in *Advance ACS Abstracts*, April 1, 1994.

0022-3549/94/1200-1222$04.50/0

performed using a Bruker 250 Nuclear Magnetic Spectrometer (Bruker Instruments, Inc., Billerica, MA).

Organic Microanalysis—Samples were analyzed (Desert Analytics, Tucson, AZ) in duplicate for carbon, hydrogen, and oxygen; cholesteryl flufenamate was also analyzed for nitrogen.

Mass Spectroscopy—Mass spectra were obtained using direct insertion probe analysis (Model 5988A mass spectrometer, Hewlett-Packard Corp., Avondale, PA). The probe temperature was programmed from 80 to 325 °C at 30 °C/min. The instrument was scanned from m/z 50 to 700.

KCl/KBr Solution—A KCl/KBr solution with a density of 1.006 g/mL was prepared by adding 17.55 mL of saturated KBr solution (d = 1.3705 g/mL) to 1 L of 0.05 M KCl solution (d = 0.9996 g/mL).

Particle Size Determination—The particle size distribution of the microemulsion was determined using laser light scattering (Model N4MD submicron particle size analyzer, Coulter Electronics, Hialeah, FL) with a 90° detection angle at 25 °C. Samples were diluted with deionized water which had been filtered through a 0.2-μm Puradisc 25PP polypropylene filter (Whatman, Maidstone, England) to obtain a sample intensity between 1.0×10^5 and 2.0×10^5 counts/s. All determinations were run with a 1000 s run time.

Ultracentrifugation—Samples were centrifuged at 37 000 rpm (average relative centrifugal field = 90000g, range = 57000g to 122000g) for a h at 5 °C using an ultracentrifuge (L8-55 ultracentrifuge with a type 75Ti fixed angle rotor, Beckman Instruments, Inc., Palo Alto, CA).

HPLC Analysis—Microemulsions were assayed by HPLC using a modification of the method of Lize et al.[14] Analyses were run with a HPLC system consisting of a Series 400 solvent delivery system, LC-75 spectrophotometric detector (Perkin-Elmer Corp., Norwalk, CT), WISP 712 automatic sample injector (Waters Assoc., Milford, MA), and SP4270 integrating recorder (Spectra-Physics, San Jose, CA). Analyses were run using a mobile phase consisting of methanol:acetonitrile:tetrahydrofuran:water (290:400:400:120) with a flow rate of 1.0 mL/min on a Zorbax C-8 column, 4.6 mm i.d. × 15 cm (Dupont, Inc., Wilmington, DE). A 25-μL sample was injected onto the column, and peaks were detected at a wavelength of 214 nm. Retention volumes were 5.6 mL for DPPC, 10.2 mL for 3, and 11.0 mL for 4.

Eutectic Mixture Preparation—Binary mixtures were prepared of cholesteryl ibuprofen:cholesteryl oleate (CI:CO), cholesteryl ibuprofen:triolein (CI:TO), cholesteryl flufenamate:cholesteryl oleate (CF:CO), and cholesteryl flufenamate:triolein (CF:TO) in weight ratios of 10:90, 20:80, 30:70, 50:50, 70:30, and 90:10. To prepare the binary mixtures, solutions of the individual lipids were prepared in chloroform. Aliquots of the chloroform solutions were transferred to test tubes and combined to achieve the appropriate weight ratios for each of the compounds. After evaporation of the chloroform under a stream of nitrogen at 45 °C, the test tubes were then placed in a 45 °C vacuum oven for 24 h. Samples were then weighed into vented DSC pans and placed in a 3 °C refrigerator for a minimum of 24 h before testing.

Differential Scanning Calorimetry—DSC studies were conducted on 15-mg samples using a TA3000 system with a DSC-20 standard cell (Mettler Instruments Corp., Hightstown, NJ). In order to study transitions below room temperature, the DSC-20 standard cell was placed in a 1.5-cub ft refrigerator (Sanyo Corp., Tokyo, Japan) set at a temperature of 3 °C. All determinations were run in triplicate. Samples were scanned from 5 to 180 °C at 5 °C/min.

Solubility in Triolein—The solubilities of 3 and 4 were determined in triolein. A 1-g sample of triolein was weighed into a 4-mL screw-cap vial. After separate excess amounts of 3 and 4 were added to the vial, the vials were allowed to mix on a rotating mixer for 7 days. Samples were removed from each vial and filtered through a 0.8-μm-polycarbonate Nucleopore filter (Nucleopore Corp., Pleasanton, CA) to remove any undissolved material and weighed into a 5-mL volumetric flask. They were then dissolved in acetonitrile:tetrahydrofuran (50:50) and assayed for amount of cholesteryl ester by HPLC as described above.

Cholesteryl Ester Synthesis—All glassware was dried in an oven at 110 °C for 24 h prior to use. Reagents were weighed into three flasks. The flasks were evacuated, purged with argon 10 times, and sealed with a rubber septum. Addition of anhydrous methylene chloride and all transfers were performed using a gas-tight syringe. In a typical synthesis, cholesterol (3.70 g, 9.6 mmol) was weighed into a 50-mL pear-shaped flask and dissolved in 35 mL of anhydrous methylene chloride. Dicyclohexylcarbodiimide (1.86 g, 9.0 mmol) was weighed into a 25-mL pear-shaped flask and dissolved in 20 mL of anhydrous methylene chloride. A sample of 1 or 2 (1.79 g, and 1.86 g, respectively, 8.7 mmol) and 4-pyrrolidinopyridine (133 mg, 0.9 mmol) were weighed into a 250-mL two-neck round-bottom flask and dissolved in 10 mL of anhydrous methylene chloride.

During the reaction, a positive pressure of argon was maintained on the reaction flask by an argon-filled balloon on one neck of the flask; the other neck was sealed with a rubber septum. The cholesterol and the dicyclohexylcarbodiimide solutions were successively added to the reaction flask and stirred with a magnetic stirrer. The reaction was then allowed to stir at room temperature under argon overnight (typically 18–24 h).

Immediately after the addition of the dicyclohexylcarbodiimide solution, and periodically throughout the reaction, samples were removed with a syringe and spotted onto a 1 × 3 in. TLC plate. The reaction was considered complete when no evidence of the cholesterol or ibuprofen starting material was evident.

After allowing the reaction to stir overnight, a white precipitate formed which was identified as dicyclohexylurea by IR and GC–MS analysis. The reaction mixture was filtered (qualitative P4 filter paper, Fisher Scientific, Pittsburgh, PA) to remove the precipitated dicyclohexylurea and evaporated to dryness under vacuum at 45 °C. The resulting residue was dissolved in 10 mL of hexane and filtered a second time to remove any undissolved material.

Cholesteryl esters (3 and 4) were purified using column chromatography. The filtrate from above was applied to a 2.5 cm diameter × 7 cm length silica gel column and eluted with hexane. 3 and 4 were the first compounds to elute from the column as verified by TLC on 20 cm × 20 cm silica gel plates. Fractions containing 3 or 4 were combined, and the hexane was evaporated under vacuum at 45 °C.

For 3, a typical yield was approximately 75%. 3 was recrystallized by dissolving in a minimum amount of hot 1-propanol (boiling point = 97.6 °C) and allowing to stand undisturbed until cooled to room temperature. The resulting product was a white amorphous solid which was then filtered under vacuum, washed with ice-cold 1-propanol, and dried in a vacuum oven at 40 °C overnight. A typical yield after recrystallization was approximately 50% overall.

For 4 a typical yield was approximately 63%. The resulting product was a white crystalline solid. A typical yield after recrystallization was approximately 60% overall.

In Vitro Cholesteryl Esterase Assay—Using the method of Heider and Boyett[15] the hydrolyses of cholesteryl oleate, cholesteryl palmitate, and cholesteryl benzoate were compared to those of 3 and 4. Cholesteryl esters are hydrolyzed using a cholesteryl ester hydrolase (from porcine pancreas). The free cholesterol is oxidized by cholesterol oxidase, liberating H_2O_2. The H_2O_2 is coupled with p-hydroxyphenylacetic acid by the action of horseradish peroxidase to yield a chromogen which is detected fluorometrically at an excitation wavelength of 325 nm and an emission wavelength of 415 nm. Solutions of each of the cholesteryl esters were prepared in 2-propanol at a concentration equivalent to approximately 25 μg/mL of cholesterol. Total cholesterol reagent contained the following: sodium phosphate buffer, pH 7.0, 0.05 M; cholesteryl ester hydrolase, 0.08 IU/mL; cholesterol oxidase, 0.08 IU/mL; horseradish peroxidase, 30 IU/mL; sodium taurocholate, 5 mM; polyethylene glycol 8000, 0.17 mM; and p-hydroxyphenylacetic acid, 0.15 mM.

In triplicate, 40-μL aliquots of the cholesteryl ester solutions were placed in separate test tubes. To each tube was added 800 μL of the total cholesterol reagent, and the contents were mixed well. The tubes were incubated at 37 °C. After incubation periods of 1, 18, and 36 h, 1.6 mL of 0.5 N NaOH was added to each tube, and the contents were mixed well. Fluorescence was determined with an excitation wavelength of 325 nm and an emission wavelength of 415 nm (Model LS-5B fluorometric spectrometer, Perkin-Elmer Corp., Norwalk, CT).

Microemulsion Preparation—Dipalmitoylphosphatidylcholine (DPPC) and cholesteryl ester (CE) were dissolved in 1-propanol in a test tube and heated to 96 °C in a circulating water bath along with six test tubes (20 mm × 150 mm) containing a 10-mL aliquot of KCl/KBr solution, d = 1.006 g/mL. A 400-μL aliquot of the DPPC/CE solution was then slowly added dropwise to each of the six KCl/KBr solutions while being vortexed. Vortexing was continued for 5 min after the addition of the DPPC/CE solution. The size of the resulting translucent microemulsion was determined using the Coulter N4MD. For a typical microemulsion, with a molar ratio of DPPC:CI 80:20, DPPC (150 mg, 0.20 mmol) and 3 (30 mg, 0.05 mmol) were dissolved

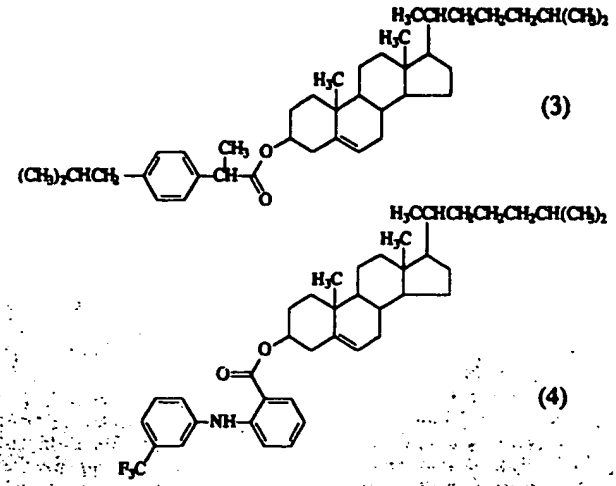


Figure 1—Structure of two cholesteryl ester prodrugs, cholesteryl ibuprofen (3) and cholesteryl flufenamate (4).

in 3 mL of 1-propanol, and 400-μL aliquots of the hot DPPC/Cl solution were added to the hot KCl/KBr solution in each of six test tubes.

After the microemulsions cooled to room temperature, they were transferred to 10.4-mL screw cap ultracentrifuge tubes (Nalge Co., Rochester, NY) and centrifuged for 1 h at 37 000 rpm. The tubes were then carefully removed and the supernatant (fraction 1) was removed with a Pasteur pipet. This fraction contains material with a density of <1.006 g/mL. The pelleted material remaining in the tube was resuspended in 2.0 mL of KCl/KBr, d = 1.006 g/mL, by briefly ultrasonicating the centrifuge tubes in a water bath.

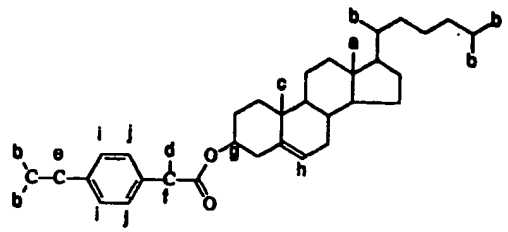
For the second ultracentrifugation, the density of the KCl/KBr solution was adjusted to d = 1.22 g/mL by adding 2.85 mL of saturated KBr solution to each tube. The microemulsions were then recentrifuged for 1 h at 37 000 rpm. The majority of the microemulsion floated to the top of the tube in a gel-like mass. This material was carefully removed with a Pasteur pipet and labeled fraction 2. Fraction 2, which contains material having a density between 1.006 and 1.22 g/mL, was used for all further studies. Pooled fractions from each of the six tubes were combined and resuspended in 0.9% sodium chloride to achieve a final volume of 10 mL. The material in the lower portion of the tube was removed along with any other material which had pelleted after the second ultracentrifugation and labeled Fraction 3. Fraction 3 contains any material with a density >1.22 g/mL.

III. Results

Using the method of Patel et al.,[12] two cholesteryl ester prodrugs, cholesteryl ibuprofen and cholesteryl flufenamate, have been synthesized. These two cholesteryl ester prodrugs, 3 and 4, have not been previously reported in the literature. The proposed structures of 3 and 4 from the preceding synthesis are shown in Figure 1. Verification of the proposed structures was performed using infrared spectroscopy, proton nuclear magnetic spectroscopy, elemental analysis, and mass spectroscopy.

Cholesteryl Ibuprofen and Cholesteryl Flufenamate Characterization—Cholesteryl ibuprofen (3β-cholest-5-enyl [α-methyl-4-(2-methylpropyl)benzeneacetate) was isolated as a waxy white amorphous powder. As the ibuprofen starting material was a racemic mixture, one would expect 3 to be a racemic product. The optical purity of 3 was not determined. The melting range determined with a capillary melting point apparatus was 114–120 °C. This wide melting range could be due to the racemic nature of the product. The peak endotherm determined with DSC was 113.7 ± 0.1 °C, with a ΔH of 51.20 ± 2.14 J/g (n = 3).

Table 1—NMR Data for Cholesteryl Ibuprofen



Position	Chemical Shift (ppm)	Multiplicity	Integration
a	0.66	singlet	3H
b	0.85–0.92	doublet of doublets	15H
c	1.00	singlet	3H
d	1.45–1.48	doublet	3H
e	2.43–2.45	doublet	3H
f	3.60–3.72	quartet	1H
g	4.55–4.68	multiplet	1H
h	5.30–5.40	multiplet	1H
i	7.06–7.09	doublet	2H
j	7.18–7.21	doublet	2H

Cholesteryl flufenamate (3β-cholest-5-enyl 2-[[3-(trifluoromethyl)phenyl]amino]benzoate) was isolated as a white crystalline solid. The melting range determined with a capillary melting point apparatus was 145–148 °C. The peak endotherm determined using DSC was 147.3 ± 0.2 °C, with a ΔH of 56.20 ± 0.40 J/g (n = 3).

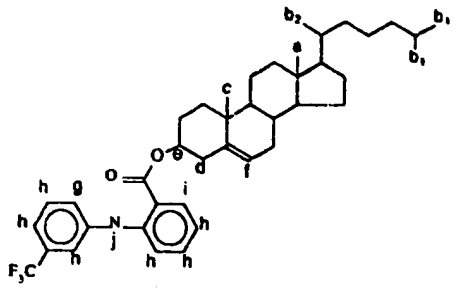
The infrared spectra of 3 and 4 were determined. The spectra show the presence of a strong sharp peak characteristic of the ester C=O stretch at 1731.5 cm^{-1} for 3 and 1676.0 cm^{-1} for 4. The C=O stretch of 4 occurs at lower wavenumber characteristic of an ortho-amino ester. A group of moderately strong peaks, at 800.3–839.6 cm^{-1} for 3 and 792.9–831.1 cm^{-1} for 4, are indicative of the ethylenic C–H bending of the Δ^5 bond of the cholesterol moiety.[16] In both spectra a strong group of peaks in the region 2800–3000 cm^{-1}, due to the asymmetric and symmetric stretching vibrations of the aliphatic C–H bonds occur in the same region as for cholesterol. For 4 a strong sharp peak at 3307 cm^{-1} is characteristic of the N–H stretch of a secondary amine.[17] However in both spectra, the O–H bond stretching of the 3β-OH of cholesterol between 3220 and 3500 cm^{-1} and between 2350 and 3300 cm^{-1} due to the carboxylic OH of ibuprofen[16] is absent. These data are consistent with the formation of 3 and 4.

The NMR spectra of 3 and 4 are summarized in Tables 1 and 2, respectively. The 3-αH signal of cholesteryl derivatives (at 3.8 ppm in cholesterol) is shifted downfield (at 4.5 ppm for 3 and 4.75 ppm for 4), proving that the adjacent alcohol has been esterified. The NMR spectrum of 3 shows 10 major groups of peaks which can be attributed to the major peaks of both the ibuprofen and cholesterol moieties. The NMR spectrum of 4 shows 11 major groups of peaks which can be attributed to the flufenamic acid and cholesterol moieties.

Organic microanalysis was performed in duplicate. Determination of C, H, and O was performed for both. In addition, N was determined for 4. The results are summarized in Table 3. The results of the elemental analysis are within 0.05% and 0.17% of the theoretical values for 3 and 4, respectively, and further supports that the reaction products are cholesteryl ibuprofen, $C_{40}H_{62}O_2$, and cholesteryl flufenamate, $C_{41}H_{54}NO_2F_3$.

The mass spectra of 3 and 4 are summarized in Tables 4 and 5, respectively. Weak molecular ion peaks are evident at m/z = 574 for 3 and m/z = 649 for 4. This is typical of most cholesteryl esters, as they will fragment into the cholesterol and acid moieties. For 3 a prominent peak at m/z

Table 2—NMR Data for Cholesteryl Flufenamate



Proton	Chemical Shift (ppm)	Multiplicity	Integration
a	0.69	singlet	3H
b1	0.85–0.86	doublet	6H
b2	0.87–0.88	doublet	3H
c	1.07	singlet	3H
d	2.46–2.49	doublet	2H
e	4.87–4.97	multiplet	1H
f	5.42–5.44	doublet	1H
g	6.82–6.85	triplet	1H
h	7.26–7.48	multiplet	6H
i	7.99–8.03	doublet	1H
j	9.69	singlet	1H

Table 3—Results of Organic Microanalysis of Cholesteryl Ibuprofen, $C_{40}H_{62}O_2$, and Cholesteryl Flufenamate, $C_{41}H_{54}NO_2F_3$

		%C	%H	%N	%O
3	Theoretical	83.56	10.87		5.57
	Determined	83.39	10.94		5.30
		83.58	10.95		5.85
	Avg	83.49	10.94		5.57
4	Theoretical	75.78	8.38	2.16	4.92
	Determined	76.11	8.27	2.02	4.63
		75.90	8.38	1.97	4.96
	Avg	76.00	8.32	1.99	4.79

Table 4—Mass Spectral Data for Cholesteryl Ibuprofen

Mass	Fragment	Relative Abundance (%)
574	M^+	1.0
368	CHOL – H_2O	100.0
353	368 – CH_3	18.8
260	368 – $C_6H_3CH_3$=CH	18.8
255	368 – 113	14.8
247	368 – $C_6H_3CH_3$=$CHCH_2$	18.5
213	255 – $CH(CH_2CH_2)$	13.2
161	IBU – CO_2H	59.7
83	113 – $2(CH_3)$	13.3
71	$CH_2CH_2CH(CH_3)_2$	15.4
57	$CH_2CH(CH_3)_2$	43.7

= 368 resulting from the cholesterol fragment and a strong peak at m/z = 161 resulting from the ibuprofen fragment give strong evidence that the reaction product is cholesteryl ibuprofen. For 4 a proninent peak at m/z = 369 is present resulting from the cholesterol fragment, suggesting a rearrangement has occurred, and a prominent peak at m/z = 281 resulting from the flufenamic acid fragment give strong evidence that the reaction product is cholesteryl flufenamate.

In Vitro Cholesteryl Esterase Hydrolysis—Table 6 shows the rates of in vitro enzymatic hydrolysis of 3 and 4 compared to those of cholesteryl oleate, cholesteryl palmitate, and cholesteryl benzoate. The rate of hydrolysis of the two cholesteryl ester prodrugs has been shown in vitro to be much

Table 5—Mass Spectral Data for Cholesteryl Flufenamate

Mass	Fragment	(%) Relative Abundance
649	M^+	5.6
369	CHOL – OH	29.7
353	CHOL – CH_3	3.3
281	280 + H^+	100.0
263	281 – H_2O	53.0
255	369 – 113 – H^+	5.1
247	369 – $C_6H_3(CH_3)$=CH	10.7
235	236 – H^+	8.1
216	235 – F	6.2
213	255 – $CHCH_2CH_2$	4.6
166	235 – CF_3	4.3
145	$C_6H_4CF_3$	20.4
83	113 – $2(CH_3)$	12.9
71	$CH_2CH_2CH(CH_3)_2$	10.6
69	CF_3	24.6
57	$CH_2CH(CH_3)_2$	19.1

Table 6—In Vitro Hydrolysis of Cholesteryl Esters with Porcine Pancreatic Cholesterol Esterase

Cholesteryl Ester	% Hydrolysis (Mean ± SD, n = 3)		
	1 h	18 h	36 h
Cholesteryl oleate	81.2 ± 5.7	100.0 ± 0.1	
Cholesteryl palmitate	100.0 ± 0.2		
Cholesteryl benzoate	87.2 ± 0.4	100.0 ± 0.2	
Cholesteryl ibuprofen	TLTQ[a]	23.4 ± 2.5	100.0 ± 0.1
Cholesteryl flufenamate	TLTQ[a]	25.7 ± 0.6	100.0 ± 0.2

[a] Too low to quantitate.

Table 7—Particle Size Distribution of Microemulsions as a Function of the Molar Ratio of Dipalmitoylphosphatidylcholine:Cholesteryl Ibuprofen

Molar Ratio DPPC:CI	% of Population[a] (Mean ± SD, n = 6)		
	<200 nm	200–1000 nm	>1000 nm
80:20	87.3 ± 12.3	11.8 ± 13.4	1.0 ± 2.4
75:25	94.2 ± 14.3	5.8 ± 14.3	0
70:30	87.2 ± 21.0	11.5 ± 21.7	1.3 ± 3.3
65:35	69.8 ± 19.1	24.5 ± 22.5	5.7 ± 13.9

[a] SDP intensity results are related to the number of particles in the size range

slower than the native cholesteryl ester, cholesteryl oleate, and cholesteryl palmitate. Complete hydrolysis of cholesteryl palmitate was observed with a 1-h incubation. Complete hydrolysis of cholesteryl oleate and cholesteryl benzoate occurred within 18 h. Complete hydrolysis of 3 and 4 was not observed until 36 h.

Lipid Composition Effects on Particle Size of the Microemulsion—Maximum cholesteryl ester prodrug loading into the microemulsion was investigated as a function of increasing particle size distribution. In Table 7, microemulsions of DPPC:CI were prepared as described above with DPPC:CI molar ratios from 80:20 to 65:35. The particle size distribution of the resulting microemulsions was determined and separated into three groups: <200, 200–1000, and >1000 nm. Microemulsions with a mean particle size of 100–150 nm were prepared. Microemulsions were prepared in groups of six (n = 6) and average results of the particle size distributions are shown. Since it was desired to obtain microemulsions with a size in the range of 100–150 nm, the population of interest was that with a mean particle size of <200 nm. To have a large percentage of particles in the range >200 nm was considered detrimental to the formulation.

The results summarized in Table 7 indicate that as the molar percent of dipalmitoylphosphatidylcholine is reduced to 65% there is a significant increase in the percentage of large

Table 8—Particle Size Distribution of Microemulsions as a Function of the Concentration of Total Lipids in 1-Propanol Solution during Preparation

Total Concentration of Lipids (mg/mL)	Percent of Population[a] (Mean ± SD, $n = 6$)		
	<200 nm	200–1000 nm	>1000 nm
30	86.2 ± 15.7	13.8 ± 15.7	0
60	87.3 ± 12.3	11.8 ± 13.4	1.0 ± 2.4
90	56.2 ± 12.8	19.7 ± 22.3	24.2 ± 21.8
120	31.7 ± 17.6	17.8 ± 22.5	50.3 ± 27.8

[a] SDP intensity results correlated to the number of particles in each size range.

particles (>200 nm) which are formed (one-way ANOVA, $p = 0.05$ level). There is no statistically significant difference in the percent of particles in the <200-nm range among the 80:20, 75:25, and 70:30 formulations (one-way ANOVA, $p = 0.05$ level). Although the 80:20 and 75:25 formulations were not statistically different, the microemulsions formed with a ratio of 75:25, on average, produced the lowest percent of large particles with only one of six trials producing particles in the range >200 nm. On the basis of these results the molar ratio of 75% dipalmitoylphosphatidylcholine to 25% cholesteryl ester was chosen for use in all future formulations.

Lipid Concentration Effects on the Particle Size of the Microemulsion—Using a molar ratio of 75% dipalmitoylphosphatidylcholine to 25% 3 a second experiment was conducted to determine what effect increasing the concentration of total lipids (DPPC + 3) in the 1-propanol solution had on the particle size distribution of the microemulsions. In Table 8, as the concentration of lipid is varied from 30 to 120 mg/mL, the number of small particles formed falls off rapidly above 60 mg/mL. Solutions of DPPC:CI were prepared in 3.0 mL of 1-propanol with total lipid concentrations ranging from 30 to 120 mg/mL. Microemulsion preparation and particle size distribution determination were as described above.

The results indicate that, as the concentration of the lipids in the 1-propanol solution is increased above 60 mg/mL, the particle size of the resulting microemulsions is adversely shifted to a larger range. While the 30 and 60 mg/mL formulations were not statistically significantly different from one another, the 90 and 120 mg/mL formulations had a significantly greater number of large particles (>200 nm). Although there was on average a small percentage (1.0 ± 2.4%) of particles in the >1000 nm range with the 60 mg/mL formulations, it was felt that this would not adversely affect the future experiments since filtration through a 0.2-μm filter would remove these particles. On the basis of these results, a total lipid concentration of 60 mg/mL was chosen for use in all future formulations.

The aqueous phase of the microemulsions was assayed for the presence of the cholesteryl ester prodrug to determine if any of the cholesteryl ester prodrug had partitioned into the aqueous phase. Microemulsions were ultracentrifuged, and the aqueous phase was removed and assayed by HPLC for either 3 or 4 as previously described above. In all cases, there was no cholesteryl ester prodrug detected in the aqueous phase.

DSC Studies—DSC studies were conducted to evaluate if a low melting point eutectic mixture could be formed between the two cholesteryl ester prodrugs and cholesteryl oleate and to determine the reduction of their melting points with triolein. The solubilities of both cholesteryl ester prodrugs were determined in triolein.

Cholesteryl oleate eutectic mixtures with 3 and 4 are shown in Figures 2 and 3. Binary mixtures of CI:CO, CF:CO, CI:TO, and CF:TO were prepared. The peak DSC endotherms for pure cholesteryl ibuprofen and cholesteryl flufenamate were determined to be 119.7 ± 0.5 and 148.0 ± 0.4 °C, respectively. For the CI:CO diagram, extrapolation of the liquid–solid curve indicates a eutectic composition of approximately 16% cholesteryl ibuprofen and 84% cholesteryl oleate with a melting point of 35.2 ± 5.0 °C ($n = 14$). Similarly for 4, the eutectic mixture composition was found to be approximately 12% cholesteryl flufenamate and 88% cholesteryl oleate with a melting point of 45.2 ± 1.7 °C ($n = 15$). The higher variability seen with the CI:CO mixtures may be due to the presence of a racemic product which results in a broader endotherm peak of 3. With the broad peak it is more difficult to determine the peak endotherm temperature accurately. This is evident in Figure 3, where the eutectic melting point does not appear to be constant (±5.0 °C). Conversely, with 4 the endotherm peak is very sharp, allowing better accuracy in determining the peak temperature, resulting in a lower variability (±1.7 °C).

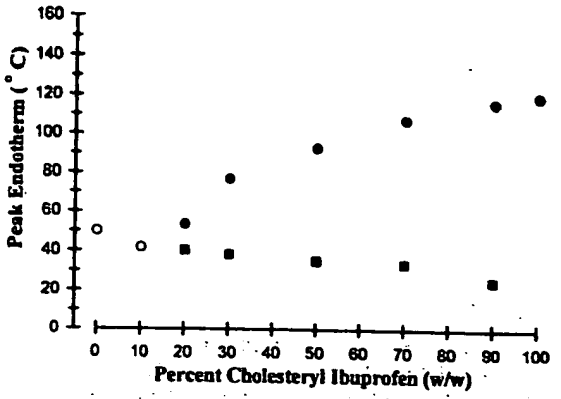


Figure 2—Phase diagram of binary mixtures of cholesteryl ibuprofen and cholesteryl oleate (Mettler TA3000 with DSC-20 standard cell, 5–180 °C at 5 °C/min: ● = cholesteryl ibuprofen, ○ = cholesteryl oleate, ■ = eutectic).

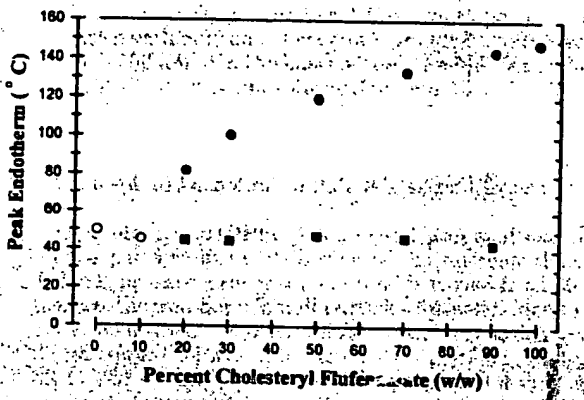


Figure 3—Phase diagram for binary mixtures of cholesteryl flufenamate and cholesteryl oleate (Mettler TA3000 with DSC-20 standard cell, 5–180 °C at 5 °C/min: ● = cholesteryl flufenamate, ○ = cholesteryl oleate, ■ = eutectic).

Binary mixtures of the two cholesteryl ester prodrugs with triolein also show a reduction of the melting point. The melting point of 3 was reduced from 119.7 ± 0.5 °C ($n = 3$) to 85.4 ± 2.0 °C ($n = 3$) at a composition of 30:70; similarly for 4, a reduction from 147.8 ± 0.6 °C ($n = 3$) to 118.3 ± 2.0 °C ($n = 3$) was observed at a composition of 30:70. At lower concentrations of the prodrugs, complete solubility occurred. Since triolein has a melting point of 11 °C and exists as a liquid at physiological temperatures, the solubility of both cholesteryl ester prodrugs was determined in triolein. The solubilities of 3 and 4 in triolein were determined to be 13.2 ± 0.2% (w/w) and 11.5 ± 0.3% (w/w), respectively.

IV. Discussion

Several methods for the preparation of cholesteryl esters are available. The methods in the literature involve the reaction of cholesterol with acid halides,[12,18-20] reaction of cholesterol with acylimidazole derivatives of fatty acids,[21] reaction of cholesteryl chloride with acids,[22] and reaction of cholesterol with acid anhydrides.[23] The direct reaction of cholesterol with a carboxylic acid using the dehydrating reagent dicyclohexylcarbodiimide in the presence of an acylation catalyst[11,24] was used because of its simplicity and reported high yields. The method of Patel[12] gave cholesteryl esters of **1** and **2** in reasonable yields. No attempt was made to optimize the reactions. The incorporation of other cholesterol derivatives into lipoproteins and phospholipid microemulsions has been carried out in a number of cases. β-Sitosteryl β-D-glucopyranoside, a plant steroid, has been incorporated into lipoproteins to study its activity against P388 leukemia cells.[25] Deforge et al.[26] incorporated a radiolabeled cholesteryl iopanate into low-density lipoprotein (LDL) to study the disposition of LDL-associated cholesteryl esters in vivo. Similarly, other cholesterol derivatives including tris-[(galactosyloxy)methyl]aminomethane-terminated cholesterol, cholesteryl nitroxide, PCMA cholesteryl oleate, nitrobenzoxadiazole fluorophore derivative of cholesteryl linoleate, and sudan cholesteryl oleate have been incorporated into lipoprotein.[3] To date, however, there have been no reports of the incorporation of a cholesteryl ester prodrug into a phospholipid microemulsion for use as a drug-delivery system.

Since cholesteryl esters are hydrophobic they would be expected not to leach out of the core of the microemulsion. This is one advantage of microemulsions over vesicles, where a hydrophilic compound incorporated in the core of the vesicle may partition into the aqueous media. In addition, the cholesteryl ester prodrug may provide a means of slowly releasing the free drug in vivo. The in vitro hydrolysis of **3** and **4** was shown to occur at a much lower rate than the native cholesteryl esters cholesteryl palmitate and cholesteryl oleate. The substrate specificity of pancreatic cholesterol esterase has been studied. It has been shown that the binding of substrate to the cholesterol esterase is a hydrophobic interaction and is dependent on the chain length and degree of unsaturation.[27,28] The usual active substrates for cholesterol esterase are long chain hydrocarbon cholesteryl esters. The aromatic ring of **3** and **4** may have a steric effect preventing and reducing the interaction with the binding site of cholesterol esterase.

Two formulation variables were investigated to determine their effect on the particle size distribution of microemulsions: (1) the ratio of phospholipid:cholesteryl ester and (2) the total concentration of lipids (DPPC and CE) in the 1-propanol solution. On the basis of the results of this study, it was determined that a formulation with a DPPC:CE ratio of 75:25 resulted in a microemulsion with the least percentage of particles in the range >200 nm. Regarding total lipid concentration, preparation of the microemulsion with a 1-propanol concentration up to 60 mg/mL (DPPC + CE) resulted in microemulsions with the least percentage of larger particles. Increasing the concentration of the total lipids in the 1-propanol solution above 60 mg/mL resulted in the formation of a greater percentage of particles in the size range >200 nm. On the basis of these results, all microemulsions for in vivo studies were prepared from an alcoholic solution with a total lipid concentration of 60 mg/mL and a DPPC:CE molar ratio of 75:25.

Another strategy for increasing the concentration of the lipids in the microemulsion would have been to increase the volume of 1-propanol solution added to the KCl/KBr solution during the preparation. The addition of a larger volume of 1-propanol would have affected the density of the KCl/KBr solution. It was desired to keep the volume of 1-propanol added to the KCl/KBr solution to a minimum. In addition, increasing the percentage of 1-propanol may have changed the solubilities of the components in the KCl/KBr. Therefore, this strategy was not investigated.

Others in the literature have reported the formation of microemulsions with higher percentages of cholesteryl ester, but in all cases these investigators were able to prepare the microemulsions at a temperature above the melting point of all components.[5,6,29,30] Since both **3** and **4** have melting points above 100 °C, this is not possible. The KCl/KBr solution would boil off at the melting points of 120 and 148 °C, for **3** and **4**, respectively. The high melting point of the cholesteryl esters and their low aqueous solubility limit the ability to achieve microemulsions with a smaller mean particle size. Upon addition of the 1-propanol solution to the KCl/KBr solution, the cholesteryl esters would precipitate out of solution. This may result in crystals which cannot be reduced in size as opposed to a low melting cholesteryl ester which would remain fluid. This study demonstrates the utility and feasibility of incorporating high melting cholesteryl ester prodrugs into a phospholipid microemulsion.

Assay of the aqueous phase of the microemulsion after ultracentrifugation was performed. No **3** or **4** was detected in the aqueous supernate. This demonstrated that the hydrophobic cholesteryl ester prodrugs do not partition out of the microemulsion. These data demonstrate the benefit of forming a hydrophobic cholesteryl ester prodrug for incorporation into a phospholipid microemulsion. This strategy overcomes one of the disadvantages which is often reported for hydrophilic compounds formulated into vesicles with an aqueous core.

Other investigators have shown that the physical state of an emulsion particle substrate has an effect on the activity of cholesterol esterase in vitro.[31] In particular, they have demonstrated that if the core of a cholesteryl ester-containing emulsion exists in a liquid state the activity of the cholesterol esterase is grater. Eutectic mixtures of cholesteryl oleate with cholesteryl ibuprofen and cholesteryl flufenamate occurred at ratios of 84:16 (w/w%) and 18:12 with melting points of 35.2 ± 5.0 and 45.2 ± 1.7 °C, respectively. This raises the possibility that a formulation with CI:CO may exhibit a faster hydrolysis of the cholesteryl ester than one with CF:CO due to a eutectic with a melting point below the physiological temperature of 37.5 °C. Triolein formulations might also have faster hydrolysis rate since both **3** and **4** are soluble in triolein at 13.2% and 11.5% (w/w), respectively. With the CI:CO, CI:TO, and CF:TO formulations one would expect the core of the microemulsion to exist as a liquid at physiological temperature. Future studies will investigate the effect of the core composition on the elimination of the cholesteryl ester prodrugs in vivo.

Two cholesteryl ester prodrugs have been synthesized and characterized. The feasibility of formulating **3** and **4** into phospholipid microemulsions has been demonstrated. The microemulsions were optimized with respect to composition and resulting particle size. The microemulsions will be used in future studies to determine the feasibility of using a cholesteryl ester prodrug formulated into a phospholipid microemulsion for use as a drug-delivery system.

References and Notes

1. Lundberg, B. In *Lipoproteins as Carriers of Pharmacological Agents*; Shaw, J. M., Ed.; Marcel Dekker: New York, 1991; pp 97–139.
2. Fuchizawa, T. *J. Disp. Sci. Tech.* **1989**, *10*, 667–691.

3. Florence, A. T.; Halbert, G. W. In *Lipoproteins as Carriers of Pharmacological Agents*; Shaw, J. M., Ed.; Marcel Dekker: New York, 1991; pp 140–175.
4. Attwood, D.; Florence, A. T. *Emulsions, Surfactant Systems, Their Chemistry, Pharmacy, and Biology*; Chapman and Hall: New York, NY, 1983.
5. Mims, M. P.; Guyton, J. R.; Morrisett, J. D. *Biochemistry* **1986**, *25*, 474–483.
6. Ginsburg, G. S.; Small, D. M.; Atkinson, D. *J. Biol. Chem.* **1982**, *257*, 8216–8227.
7. Nishikawa, O.; Yokoyama, S.; Kurasawa, T.; Yamamoto, A. *J. Biochem.* **1986**, *99*, 295–301.
8. Sklar, L. A.; Craig, L. F.; Pownall, H. J. *J. Biol. Chem.* **1981**, *256*, 4286–4292.
9. Via, D. P.; Craig, I. F.; Jacobs, G. W.; Van Vinkle, W. B.; Charlton, S. C.; Gotto, A. M.; Smith, L. C. *J. Lipid Res.* **1982**, *23*, 570–576.
10. Berthod, A.; Jin, H. L.; Beesley, T. E.; Duncan, J. D.; Armstrong, D. W. *J. Pharm. Biomed. Anal.* **1990**, *8*, 123–130.
11. Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Longman Scientific and Technical Div., Wiley: New York, 1989; p 399.
12. Patel, K. M.; Sklar, L. A.; Currie, R.; Pownall, H. J.; Morrisett, J. D. *Lipids* **1979**, *14*, 816–818.
13. Gibson, H. W. *Mol. Cryst. Liq. Cryst.* **1974**, *27*, 43–53.
14. Lize, J. M.; Postaire, E.; Prognon, P.; Delattre, J.; Pradeau, D. *Pharm. Acta Helv.* **1988**, *63*, 239–240.
15. Heider, J. G.; Boyett, R. L. *J. Lipid Res.* **1978**, *19*, 514–518.
16. Gibbons, G. F.; Mitropoulos, K. A.; Myant, N. B. *Biochemistry of Cholesterol*; Elsevier Biomedical Press: New York, 1982.
17. El Hinnawi, M. A.; Najib, N. M. *Int. J. Pharm.* **1987**, *37*, 175–177.
18. Szabo, J. A.; Zoltai, A. I.; Motika, G.; Agocs, P. M.; Miklos, F. *Acta Phys. Chem.* **1979**, *25* (3–4), 167–71.
19. Helmich, O.; Hradec, J. *J. Labelled Compds. Radiopharm.* **1981**, *18* (5), 747–754.
20. Saroja, M.; Kaimal, T. N. B. *Synth. Commun.* **1986**, *16* (1), 1423–1430.
21. Grover, A. K.; Cushley, R. J. *J. Labelled Compd. Radiopharm.* **1979**, *16*, 307–313.
22. Heilbron, I. M.; Morton, R. A.; Sexton, W. A. *J. Chem. Soc.* **1928**, *1*, 47–51.
23. Lentz, B. R.; Barenholz, Y.; Thompson, T. E. *Chem. Phys. Lipids* **1975**, *15*, 216–221.
24. Dang, Q. Q.; Rogalle, P.; Puechmaurel, B.; Jollet, P.; Negre, A.; Vieu, C.; Salvayre, R.; Doust-Blazy, L. *Nato ASI Ser., Ser. A* **1988**, *159* (lipid disord.), 499–502.
25. Seki, J.; Okita, A.; Watanabe, M.; Nakagawa, T.; Honda, K.; Tatewaki, N.; Sugiyama, M. *J. Pharm. Sci.* **1985**, *74*, 1259–1264.
26. Deforge, L. A.; Degalan, M. R.; Ruyan, M. K.; Newton, R. S.; Counsell, R. E. *Nucl. Med. Biol.* **1992**, *19*, 775–782.
27. Kinnunen, P. M.; Klopf, F. H.; Bastiani, C. A.; Gelfman, C. M.; Lange, L. G. *Biochemistry* **1990**, *29*, 1648–1654.
28. Gandarias, J. M.; Lacort, M.; Martinez, M. J.; Fernandez, C. J.; Ochoa, B. *Steroids* **1987**, *49*, 403–417.
29. Craig, I. F.; Via, D. P.; Bette, C. S.; Sklar, L. A.; Mantulin, W. W.; Gotto, A. M.; Smith, L. C. *J. Biol. Chem.* **1982**, *257*, 330–335.
30. Parks, J. S.; Martin, J. A.; Johnson, F. L.; Rudel, L. L. *J. Biol. Chem.* **1985**, *260*, 3155–3163.
31. Lundberg, B. B.; Rothblat, G. H.; Glick, J. M.; Philips, M. C. *Biochim. Biophys. Acta* **1990**, *1042*, 301–309.

Acknowledgments

Financial support for this work was provided by the French/Gano/Kilmer/Pollard Predoctoral Fellowship of the Philadelphia College of Pharmacy and Science and by the Parke-Davis Pharmaceutical Research Division of the Warner-Lambert Co. Thanks to Jeffery Adams of Drexel University Chemistry Department, Philadelphia, PA, for running NMR analysis and National Medical Services, Willow Grove, PA, for performing mass spectral analysis.

STIC-ILL

Abstracts only
12-0

From: Berman, Alysia
Sent: Saturday, March 17, 2001 6:45 PM
To: STIC-ILL
Subject: 09/423715

Please get the following journal articles and have them delivered to my mailbox in CM1-3B19:

Aoki, S.; Okamoto, A.; Danjo, K.; Sunada, H.; Otuka, A. 'Compatibility of ibuprofen and ethenzamide' Drug Dev. Ind. Pharm. (1997) 23(6):561-565.

Murtha, John L.; Ando, HOward Y. 'Synthesis of hte cholesteryl ester prodrugs chloesteryl ibuprofen and cholesteryl flufenamate and their formulation into phospholipid microemulsions' J. Pharm. Sci. (1994) 83(9):1222-8.

Goroshevich, R. V.; Kosmynin, A. S.; Rozhanskaya, A. E.; Tkachenko, M. L. 'Physicochemical study of drug binary systems' Khim.-Farm. Zh. (1992) 26(2):73-6.

Mura, P.; Liguori, A.; Bramanti, G. 'Solid dispersions of ibuprofen in urea. Effects of urea on dissolution' Farmaco, Ed. Prat. (1986) 41(12):377-87.

Rogers, J. A.; Anderson, A. J. 'Physical characteristics and dissolution profiles of ketoprofen-urea solid dispersions' Pharm. Acta Helv. (1982) 57(10-11):276-81.

Thank you.

Alysia Berman
Patent Examiner
CM1-3D06
(703) 308-4638
1619

Article title	Compatibility of ibuprofen and ethenzamide
Article identifier	0363904597002348
Authors	Aoki_S Okamoto_A Danjo_K Sunada_H Otuka_A
Journal title	Drug Development and Industrial Pharmacy
ISSN	0363-9045
Publisher	Marcel Dekker
Year of publication	1997
Volume	23
Issue	6
Supplement	0
Page range	561-565
Number of pages	5
User name	Adonis
Cost centre	Development
PCC	$12.00
Date and time	Tuesday, March 20, 2001 10:40:56 AM

Drug Development and Industrial Pharmacy, 23(6), 561-565 (1997)

RESEARCH PAPER

Compatibility of Ibuprofen and Ethenzamide

S. Aoki,[1] A. Okamoto,[1] K. Danjo,[2] H. Sunada,[2] and A. Otuka[2]

[1]*Research Laboratory, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Ohmiya-shi, Saitama 330, Japan*
[2]*Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468, Japan*

ABSTRACT

The compatibility of ibuprofen and various drugs was investigated by thermal analysis. The results showed a lower melting point with many drugs. The compound of ibuprofen and ethenzamide was selected for detailed compatibility investigation. First, a ratio composition of a eutectic of ibuprofen and ethenzamide was estimated. A ratio composition of a eutectic of ibuprofen and ethenzamide of weight ratio 3:2 was suggested, and its melting point was approximately 56°C. Further, we investigated crystallization by powder x-ray diffraction. The resulting powder x-ray diffraction pattern of the compound that was heat treated was almost the same as that of the physical mixture, indicating that the crystallinity of ibuprofen and ethenzamide were not affected by the heat treatment. Next, we investigated the chemical stability of ibuprofen, ethenzamide, and a small amount of various excipients in capsule form, stored under conditions of 65°, 50°, and 40°C. It was established that ibuprofen and ethenzamide are stable. However, it was found that there is a remarkable delay of dissolution speed under conditions above 50°C.

INTRODUCTION

Ibuprofen, a nonsteroidal anti-inflammatory drug, has been considered effective and safe. Recently, however, combination drugs incorporating ibuprofen have been developed. For this reason, physical and chemical compatibility studies between ibuprofen and such drugs are considered very important.

Najib (1-3) and Mura (4) reported that the solid dispersion of ibuprofen in polyvinylpyrrolidone or urea results in an increase in the in vitro release of the ibuprofen. Imai (5) and Acarturk (6) reported the for-

mulation of ibuprofen using gelatin. Gordon (7) reported on the interaction between ibuprofen and stearates which were simple eutectics. While some studies have investigated the dissolution and absorption increase, papers reporting compatibility studies have been relatively few.

There have been many compatibility studies based on thermal analysis [Botha (9–12), Cotton (13), Signoretti (14), Hartauer (15), Gunawan (16)]. However, papers reporting the relation between thermal analysis and chemical stability are few.

Considering this, we investigated the compatibility of ibuprofen and various other drugs by differential scanning calorimetry (DSC). We chose a compound of ibuprofen and ethenzamide because the mixture melts at a very low temperature. We clarified the eutectic composition and investigated the eutectic mixture in regard to the crystallization, the dissolution, and the long-term stability.

EXPERIMENTAL

Materials

The following substances were used: ibuprofen (IBP), acetaminophen, ethenzamide (ETH), carbinoxamine maleate, anhydrous caffeine, thiamine nitrate, chlorpheniramine maleate, ascorbic acid, dextromethorphan hydrobromide, potassium guaiacolsulfonate, noscapine, bisibuthiamine, riboflavin, and bromovalerylurea. All of the substances were of pharmaceutical grade.

For measurement of powder x-ray diffraction, IBP and ETH at a weight ratio of 3:2, were used. After being melted in the compound, they shattered upon cooling.

We prepared capsules for stability tests. The formulations are shown in Table 1.

Table 1

Formulation Used in the Stability Studies

Materials	Amount (w/w%)
Ibuprofen	22.9
Ethenzamide	14.6
Anhydrous caffeine	8.0
Bromovaleryiurea	31.8
Microcrystalline cellulose	12.5
Light anhydrous silicic acid	1.6
Magnesium stearate	1.3
Talc	7.3

Thermal Analysis

A 1090B Thermal Analyzer (Dupon Co.) and a thermal analysis system, TAS100 (Rigaku), were used for the thermal analysis. Thermograms were obtained by heating at a constant rate of 5°C per minute. And in case of the compound of IBP and ETH, we calculated a quantity of heat up to 60°C.

Powder X-ray Diffractometry

The powder X-ray diffraction patterns were obtained on a Rigaku Denki Geiger RAD-C, using CuK α radiation, over a range of 2θ = 3°–40° (speed 4°/min) at room temperature.

Stability Studies

The quantities of IBP and ETH were measured by the high-performance liquid chromatography (HPLC) method. The samples were stored at 65°, 50°, and 40°C.

RESULTS AND DISCUSSION

DSC of the Compound of IBP and Other Drugs

In this research, because of the very low melting point of the compound, stabilization problems and problems associated with the manufacturing process were considered likely to occur. The various melting points of compounds are shown in Table 2.

Various drugs were classified on the basis of compatibility with IBP into three types, as mentioned above. Drugs of group 1 were made eutectic with IBP, and the mixture melting point was about 56°–58°C. These mixtures have been considered to present problems in the manufacturing process and the stabilization. The melting point of mixture of IBP and drugs of group 2 were 60°–70°C, and these are probably suitable. Drugs of group 3 were compatible with IBP.

Then, we selected ETH from these drugs and conducted a further detailed examination of a ratio composition of a eutectic compound of IBP and ETH. A thermal analysis result is shown in Fig. 1. The samples consisted of a mixture of IBP and ETH in a weight ratio of 1:9–9:1. Gorden (7) suggested that stearic acid, stearyl alcohol, calcium stearate, and magnesium stearate made a simple eutectic with IBP, and estimated a ratio composition of the eutectic compound from an

Table 2

Melting Point of Mixture of IBP with Other Drugs

	m.p. (°)	Mixture m.p. (°)	Group
Ibuprofen	76	—	—
Acetaminophen	169	75.9	3
Ethenzamide	129	55.9	1
Carbinoxamine maleate	119	58.3	1
Chlorpheniramine maleate	134	62.7	2
Dextromethorphan hydrobromide	114	65.8	2
Noscapine hydrochloride	175	65.9	2
Potassium guaiacolsulfonate	252	75.6	3
Anhydrous caffeine	236	70.9	3
Thiamine nitrate	209[a]	75.1	3
Bisibuthiamine	151[a]	57.0	1
Riboflavin	297[a]	75.4	3
Ascorbic acid	193[a]	75.1	3
Bromovalerylurea	151	66.1	2

[a]Temperature of decomposition.

endothermic phase transition of the compound that further changes the ratio mix of stearates and IBP. We also estimated the ratio composition of the eutectic of IBP and ETH according to this method. It is shown in Fig. 2. The ratio composition of a eutectic was suggested to be 1:4, based on Fig. 2.

However, the results of the thermogram of No. 3 in Fig. 1 show a ratio composition different from the ratio composition of the eutectic. Because of this, there was thought to be a problem with the precision of estimation. We assume that the ratio composition of the eutectic forms a thermal analysis result of the sample of the ratio composition which is quite different from the ratio composition of the eutectic in the method used by Gorden (7).

Due to this problem, we tried to estimate the ratio composition of the eutectic using a method which measures an endothermic quantity of the eutectic's origin. This is, the endothermic peak from each thermogram of IBP and ETH up to 60°C is not admitted. Consequently, we think that a quantity of endotherm of a compound up to 60°C is the eutectic's origin. Accordingly, it is thought that the ratio composition of the eutectic have the highest endotherm up to 60°C.

The relationship between the quantity of endotherm up to 60°C and the composition ratio for each compound is shown in Fig. 3. It was suggested that the composition ratio of the eutectic was 3:2 in terms of weight ratio from a quantity of heat up to 60°C, and that the highest weight ratio was IBP:ETH at 60:40.

Crystallization of IBP, ETH, and Their Compound

The results of powder x-ray diffraction are shown in Fig. 4. The samples were IBP, ETH, and their compound weight ratio of IBP:ETH = 3:2) and a heat-treated sample of the compound. Imai (5) and Acarturk (6) reported that the crystallization of IBP produced little change, in the case of changing the method of mixing for making a compound with low molecular gelatin. The diffraction peaks in the compound coincided, which added the diffraction peak of IBP to that of ETH. And the diffraction pattern of the heat-treated sample was almost the same as that of the compound. In the case of IBP and ETH, the crystallization of IBP and ETH showed no change.

Stabilization of IBP and ETH

We investigated the stabilization of IBP and ETH using the sample described in Table 1: (i) stored at 50°C for 2 months; (ii) at 65°C for 1 month; (iii) and at 40°C for 3 months.

A storage temperature of 65°C is higher than the melting point of the eutectic of IBP and ETH. Conse-

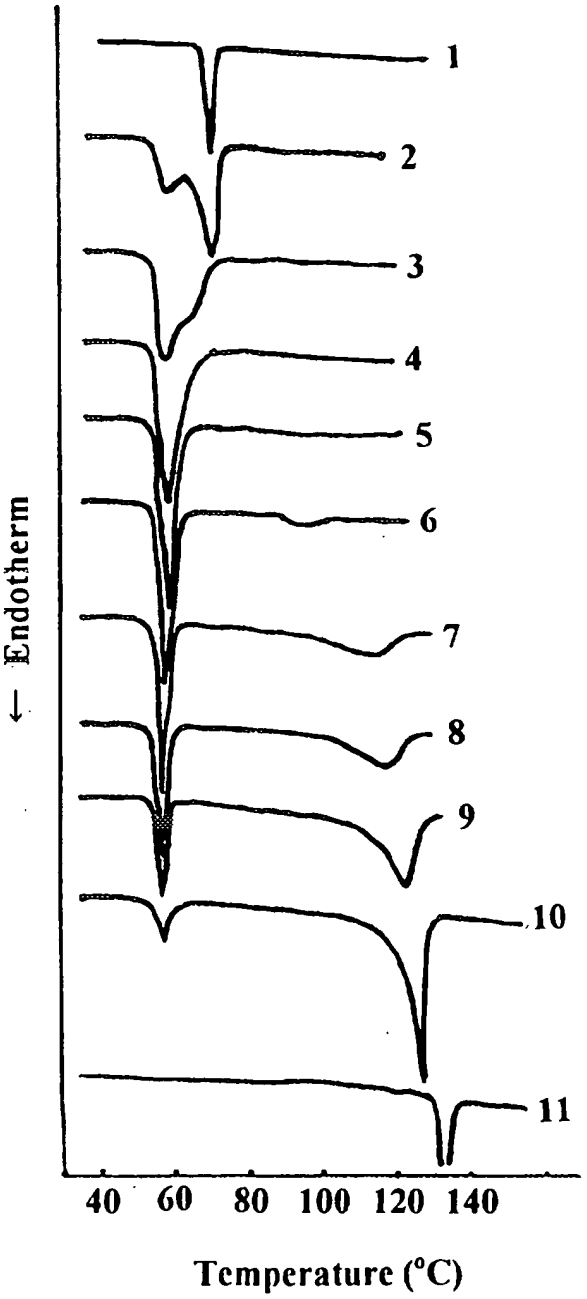


Figure 1. Thermograms of IBP-ETH system: IBP (1), 90% IBP + 10% ETH (2), 80% IBP + 20% ETH (3), 70% IBP + 30% ETH (4), 60% IBP + 40% ETH (5), 50% IBP + 50% ETH (6), 40% IBP + 60% ETH (7), 30% IBP + 70% ETH (8), 20% IBP + 80% ETH (9), 10% IBP + 90% ETH (10), ETH (11).

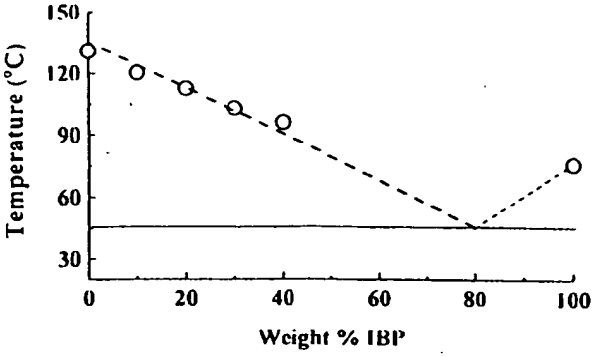


Figure 2. Phase diagram of IBP-ETH.

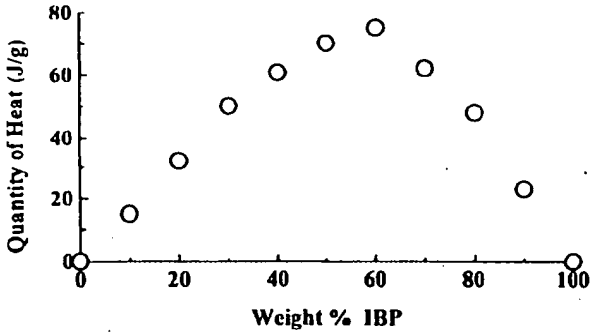


Figure 3. Quantity of heat up to 60°C of IBP and ETH mixture.

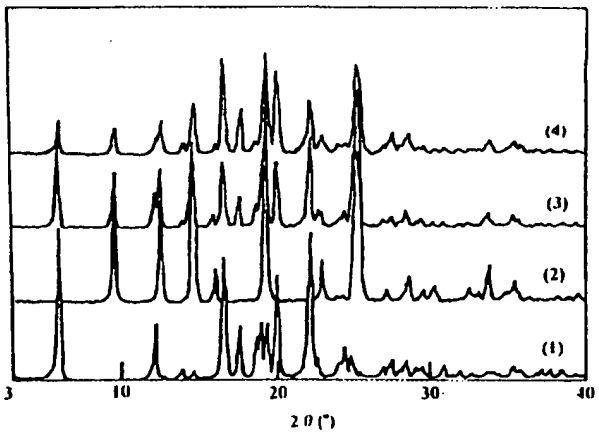


Figure 4. Powder x-ray diffraction of IBP (1), ETH (2), physical mixture IBP:ETH = 3:2 (3), and melted granulation of IBP:ETH = 3:2 (4).

Table 3

Stability of IBP and ETH

	65°C, 30 Days	50°C, 60 Days	40°C and 75% RH, 180 Days
IBP (%)	93.0	99.3	100.0
ETH (%)	99.0	102.7	99.2

quently, it was thought that samples were in the same state as that of excipients when dispersion of a melted object of the eutectic occurs.

A storage temperature of 50°C is less than the melting point of the eutectic compound. However, the sample displayed a semimelted state (an ointment-like state) because the temperature was very close to the melting point.

A sample with an inflected outside appearance was not admitted at 40°C.

As a result, we measured a quantity of the sample of IBP and ETH (Table 3), including stability on the condition that we stored it wholly. Even though IBP and ETH demonstrated a eutectic form, the chemical stabilization of their complex showed no change.

REFERENCES

1. N. M. Najib, M. Suleiman, and A. Malakh, Int. J. Pharm., 32, 229-236 (1986).
2. M. A. El-Hinnawi and N. M. Najib, Int. J. Pharm., 37, 175-177 (1987).
3. N. M. Najib, M. A. El-Hinnawi, and M. Suleiman, Int. J. Pharm., 45, 139-144 (1988).
4. P. Mura, A. Liguori, and G. Bramanti, Farm. Ed. Part., 41, 377-387 (1986).
5. T. Imai, S. Kimura, T. Iijima, T. Miyosi, M. Ueno, and M. Otagiri, J. Pharm. Pharmacol., 42, 615-1619 (1990).
6. F. Acarturk, S. Takka, I. Agaabeyoglu, Y. Ozkan, and R. Simsek, Pharmazie, 48, 761-763 (1993).
7. R. E. Gorden, C. L. Vankoevering, and D. J. Reits, Int. J. Pharm., 21, 99-105 (1984).
8. J. L. Ford and P. Timmins, *Pharmaceutical Thermal Analysis*, Ellis Horwood, 1989, pp. 238-247.
9. S. A. Botha, J. L. Du Preez, and A. P. Lotter, Drug Dev. Ind. Pharm., 12, 811-827 (1986).
10. S. A. Botha, J. L. Du Preez, and A. P. Lotter, Drug Dev. Ind. Pharm., 13, 345-345 (1987).
11. S. A. Botha, J. L. Du Preez, and A. P. Lotter, Drug Dev. Ind. Pharm., 13, 1197-1215 (1987).
12. S. A. Botha and A. P. Lotter, Drug Dev. Ind. Pharm., 15, 415-426 (1989).
13. M. L. Cotton, D. W. Wu, and E. B. Vadas, Int. J. Pharm., 40, 129-142 (1987).
14. E. C. Signoretti, C. D. Sena, A. Dell'Utri, and S. Alimonti, Drug Dev. Ind. Pharm., 14, 1167-1184 (1988).
15. K. J. Hartauer, J. K. Guillory, Drug Dev. Ind. Pharm., 17, 617-630 (1991).
16. Gunawan Indrayanto, Mugihardjo, and R. Handayani, Drug Dev. Ind. Pharm., 20, 911-920 (1994).

337,251

STIC-ILL

3B19

From: Berman, Alysia
Sent: Saturday, March 17, 2001 6:45 PM
To: STIC-ILL
Subject: 09/423715

Please get the following journal articles and have them delivered to my mailbox in CM1-3B19:

Aoki, S.; Okamoto, A.; Danjo, K.; Sunada, H.; Otuka, A. 'Compatibility of ibuprofen and ethenzamide' Drug Dev. Ind. Pharm. (1997) 23(6):561-565.

Murtha, John L.; Ando, HOward Y. 'Synthesis of hte cholesteryl ester prodrugs chloesteryl ibuprofen and cholesteryl flufenamate and their formulation into phospholipid microemulsions' J. Pharm. Sci. (1994) 83(9):1222-8.

Goroshevich, R. V.; Kosmynin, A. S.; Rozhanskaya, A. E.; Tkachenko, M. L. 'Physicochemical study of drug binary systems' Khim.-Farm. Zh. (1992) 26(2):73-6.

Mura, P.; Liguori, A.; Bramanti, G. 'Solid dispersions of ibuprofen in urea. Effects of urea on dissolution' Farmaco, Ed. Prat. (1986) 41(12):377-87.

Rogers, J. A.; Anderson, A. J. 'Physical characteristics and dissolution profiles of ketoprofen-urea solid dispersions' Pharm. Acta Helv. (1982) 57(10-11):276-81.

Thank you.

Alysia Berman
Patent Examiner
CM1-3D06
(703) 308-4638
1619

agl - 396.8. P4933

3/19

agl
3/22
100

7
63

PHARMACEUTICA ACTA HELVETIAE

Physical Characteristics and Dissolution Profiles of Ketoprofen-urea Solid Dispersions

By *J. A. Rogers* and *A. J. Anderson*

Summary

Solid dispersions of ketoprofen-urea were analyzed by differential thermal analysis and the thaw-melt technique. The phase diagram has shown that this system is characterized as a simple eutectic mixture with a eutectic composition of 90% ketoprofen and 10% urea. Dissolution studies of constant surface pellets have indicated an increased dissolution rate for solid dispersion formulations compared with pure ketoprofen or physical mixtures of ketoprofen-urea. An increase in the pH increased the dissolution rate of all formulations by the same factor but an increase in temperature increased the dissolution rate of the solid dispersions by a smaller factor than for the other two formulations. The results are interpreted to suggest that particle-size reduction is mainly responsible for the higher dissolution rate of the solid-dispersion system but a concentration of rapidly dissolving urea in the stationary layer may make a minor contribution to the dissolution rate.

Introduction

The propionic acid group of anti-inflammatory agents has gained considerable importance as a substitute for the steroids. Being weak acids, their solubility and, hence, their dissolution properties are expected to vary with the pH of the environment. However, at gastric pHs their solubilities are very low (less than 0.02%), thus, they are prime candidates for formulation studies designed to improve their dissolution characteristics from solid dosage forms. A widely accepted member of this group is ketoprofen.

Among the various approaches to improve the dissolution of drugs [1] the preparation of solid dispersion systems has often proven to be very successful [2]. Essentially, solid dispersions reduce a drug's particle size by increasing the surface area available to the dissolution medium. There are various types of solid dispersions which can be formed [2] and simple eutectics often yield the finest particle size [3] although where solid solution formation is possible the drug exists in the molecular state in the mixture with the carrier.

The aim of the present study is to demonstrate the feasibility of solid dispersion formation of ketoprofen using urea as a carrier, report on the physical characteristics of the dispersions formed and to evaluate the solid dispersions for their dissolution properties.

Experimental

Preparation of Solid Dispersions

Solid dispersions were prepared by the solvent method. Ketoprofen[1] and urea[2] were combined at various ratios in methanol[3], stirred and evaporated to dryness over gentle heat. The solidified mass was subsequently stored in a desiccator until crystallization was complete which in some instances took several weeks. The coprecipitate was then finely ground, sieved[4] then stored in the desiccator until required for use.

Preparation of Physical Mixtures

Physical mistures of drug and carrier were prepared by mechanically mixing the two substances using a mortar and pestle. The mixtures were also sieved[4] prior to use.

Differential Thermal Analysis (DTA)

The differential thermal analyzer[5] was calibrated using indium[6], tin[6], ethyl carbamate[7] and naphthalene[8]. The weights of reference material (adsorption alumina) and sample were 15 mg in all cases. Heating rate was set at 10°C/min, the differential temperature sensitivity was 0.3°C/in. and the reference temperature sensitivity was 13.7°C/in. Heats of fusion were determined from the areas under the endothermic peaks (planimeter and triangulation methods) and the corresponding calibration coefficients were obtained from the fusion temperatures (taken at the peak of the endothermic event) and the calibration curve [4]. Averages of duplicate thermograms were used for all calculations.

---

[1] Poulenc Ltd., Montreal.
[2] Fisher Scientific Co., N. J., USA, Certified A.C.S.
[3] Fisher Scientific Co., N. J., USA, Reagent Grade.
[4] US Standard Sieve, 80 mesh.
[5] Fisher Thermalyzer, Model 370, Fisher Scientific Co., Pittsburgh, Penn.
[6] Calorimetric Standards, Fisher Scientific Co., N.J., USA.
[7] BDH Chemical Ltd., Reagent Grade, Poole, England.
[8] BDH Chemical Ltd., Liquid Scintillation Grade, Poole, England.

Phase Diagram

The thaw-melt technique was employed using the Mettler FP-52 furnace melting point method[9] and equipped with an automatic temperature-programmable micro furnace. The heating rate was set at 3°C/min and starting about 10°C below the predetermined thaw-temperature accurate values of the thaw and melt temperatures were recorded.

Dissolution Rate Studies

Constant surface area pellets of ketoprofen and ketoprofen-urea solid dispersions or physical mixtures at the eutectic composition were formed by compression of a fixed weight of material at 27.6 MN/m^2 in a 1.59 cm circular punch and die[10]. The pellets were gently dusted, weighed and stored in a desiccator until ready for use.

A USP dissolution apparatus[11] was used. Pellets were placed in the baskets and lowered to a depth of 5.0 cm from the bottom of the dissolution vessel. The baskets were rotated at a constant speed of 50 ± 1 r.p.m. Studies were conducted in 600 ml of HCl acidified water at pH 2.0 or pH 5.0 and at 27 or 37°C. Aliquots of 5.0 ml were removed periodically and assayed spectrophotometrically[12] at 255 nm after which the sample was returned to the dissolution vessel. Concentrations of ketoprofen in solution were determined from a calibration curve.

Solubility Studies

The solubilities of ketoprofen were determined at pH 2.0 at 25 and 37°C in HCl acidified water or urea solutions. Excess drug was added to the aqueous phase in screw-capped vials, placed in a shaker water-bath[13] and equilibrated over a 24-hour period. Subsequently, supernatant was filtered[14] then diluted and assayed spectrophotometrically[12]. Molar solubilities, determined in triplicate and averaged, were obtained from a previously prepared calibration curve.

Results

The DTA thermograms of ketoprofen-urea solid dispersions and pure ketoprofen are depicted in Figure 1. The thermograms are characterized by two endothermic peaks over the range of 10–70% keto-

[9] Mettler Instrument Corp., Box 100, Princeton, N. J., USA.
[10] Fred S. Carver, Inc., Summit, N. J., USA.
[11] H. G. Kalish Co. Ltd., Montreal.
[12] Beckmann Model DU Spectrophotometer, Anaheim, Ca., USA.
[13] Dubnoff Metabolic Shaker, Precision Scientific, Chicago, Ill., USA.
[14] Millipore Swinney Filter Adapter, 0.45-μm filter pad, Bedford, Mass., USA.

Figure 1

Differential thermograms of solid dispersions of ketoprofen-urea and pure ketoprofen

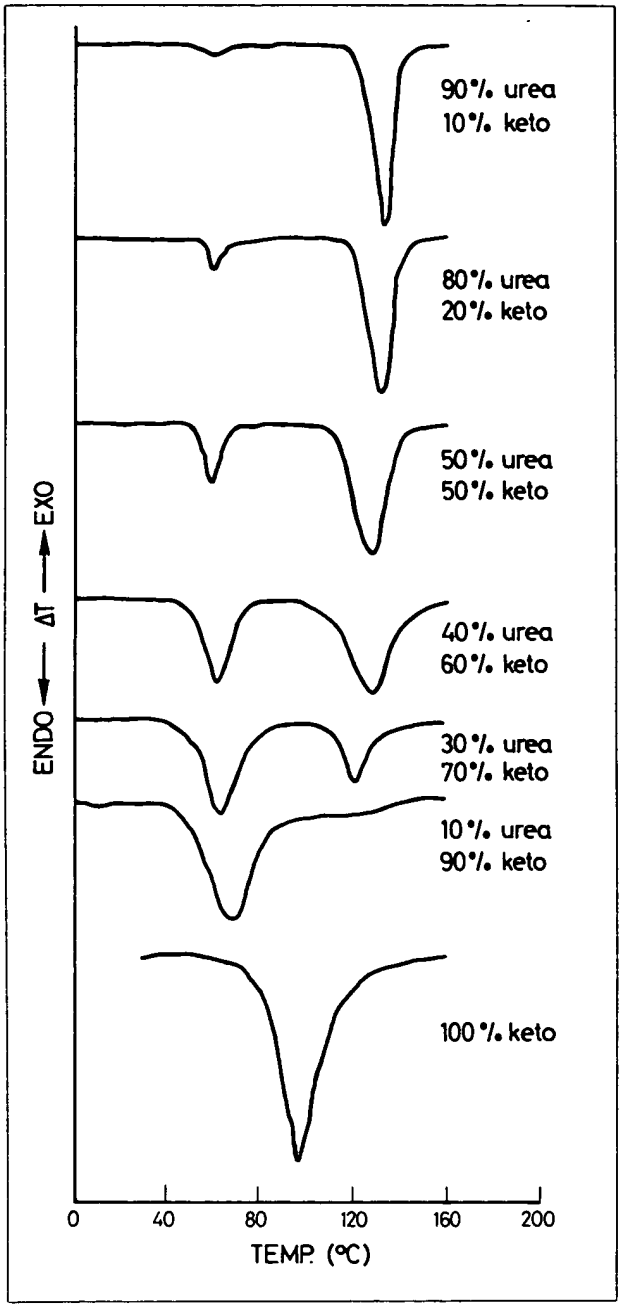


Table 1

Heats of fusion and fusion temperatures of ketoprofen-urea solid dispersions

Ketoprofen composition (%)	ΔH_F (cal/g)	Fusion temp. (°C)
10	1.07	60.0
20	2.69	61.3
50	8.73	59.3
60	10.36	62.2
70	12.60	65.0
90	17.70	69.0
100	25.08	96.0

profen corresponding to a ketoprofen-rich composition and a urea peak, respectively. The endotherms for urea gradually shift to lower temperatures as the urea content of the solid dispersion diminishes and at 10% urea its endotherm vanishes. At 95% ketoprofen and 5% urea (not shown) the single endothermic peak displayed a shoulder suggesting superposition of two peaks.

Heats of fusion (ΔH_F) and fusion temperatures for some of the compositions are given in Table 1. A linear increase in ΔH_F is observed from 10% to 70% ketoprofen whereas a nonlinear rapid increase in ΔH_F is found between 70 and 100% ketoprofen. At the same time, the fusion temperature undergoes only a slight increase, even up to 90% ketoprofen. A comparison of the physical properties of pure urea (ΔH_F = 49.10 cal/g, m.p. = 137° C [5]) with those of pure ketoprofen (Table 1) indicates that only slight contamination of one by the other considerably diminishes the energy states of the solids.

The eutectic composition of ketoprofen-urea solid dispersion was found from both DTA and the melting point method to be 90% ketoprofen and 10% urea. The phase diagram for ketoprofen-urea compositions is shown in Figure 2 and is typical of a simple eutectic mixture [6, 7]. At 95% ketoprofen the sample started to melt at a temperature higher than the eutectic temperature which indicates, together with the DTA results, the existence of solid solution formation.

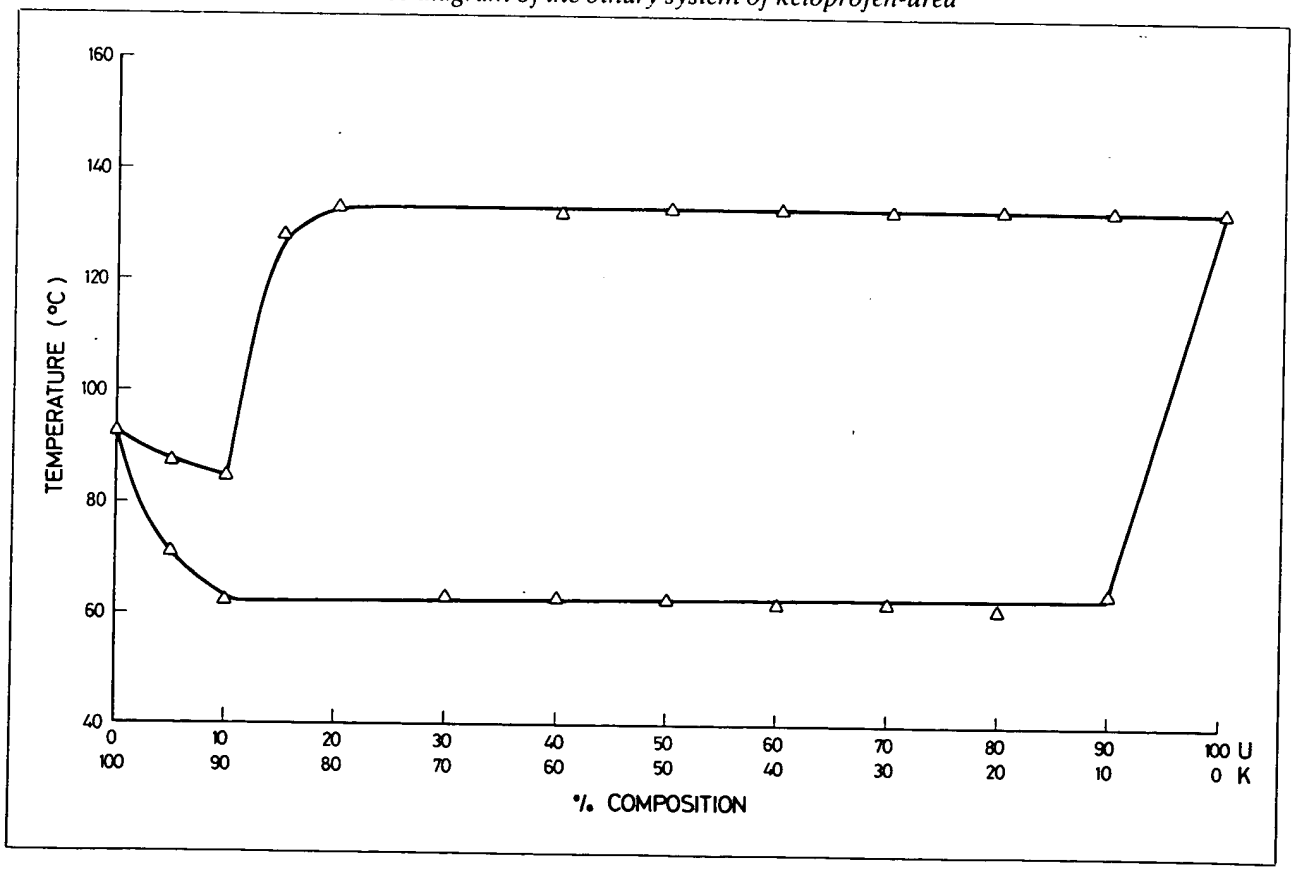
Dissolution data for constant surface non-disintegrating pellets are plotted in Figure 3 for solid dispersions at two pH values and at two temperatures. The curves are linear over the range with a correlation coefficient of 0.997 or higher in all plots. Results of the dissolution of pellets of physical mixtures of ketoprofen and urea and pure ketoprofen also produced similar linear relationships. Apparent and intrinsic dissolution rate constants were calculated from

$$F_t = \frac{K_i S t}{V} \qquad (1)$$

where the slope of a plot of the fraction of ketoprofen dissolved, F_t, against time, t, yields the apparent dissolution rate constant, $k_{app} = K_i S/V$ where K_i is the intrinsic dissolution rate constant, S is the surface area of the pellet and V is the volume of dissolution medium. Comparisons of the dissolution rate data are shown in Table 2 where it can be seen that solid dispersions of ketoprofen-urea give an increased dissolution rate over pure ketoprofen or physical mixtures of ketoprofen and urea. As expected for a weak acid drug, an increase in the dissolution rate occurs with an increase in pH or temperature. It is also clearly seen from the energetics of the dissolu-

Figure 2

Phase diagram of the binary system of ketoprofen-urea

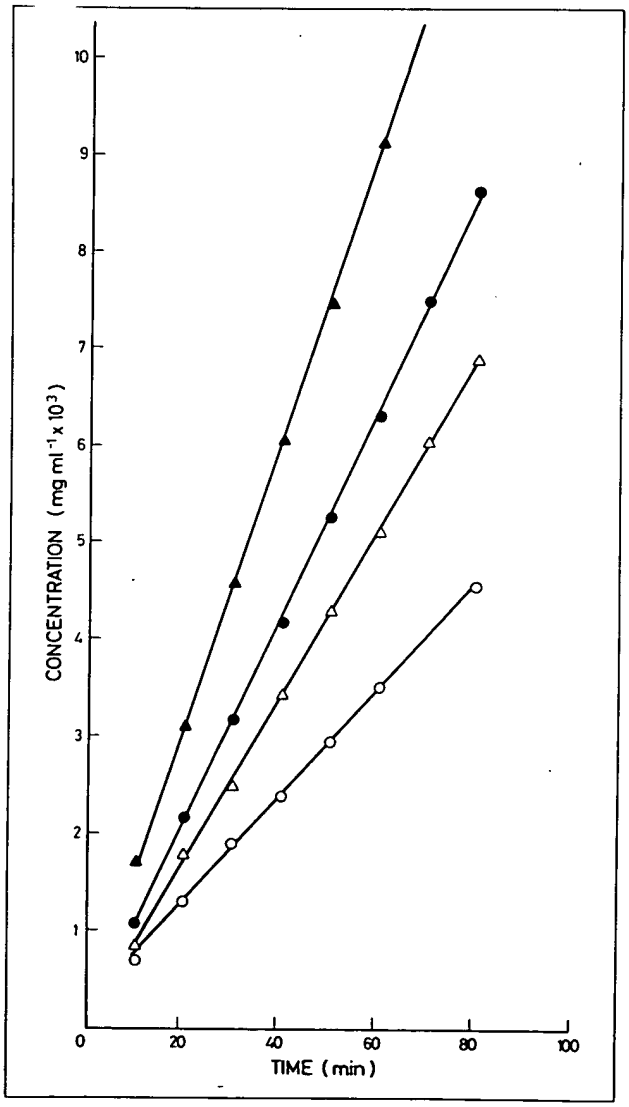


tion process (Table 3) that solid dispersions of ketoprofen-urea exist in a lower energy state than do the other forms of ketoprofen. Overall, solid dispersions yield a 60-% increase in the dissolution rate of ketoprofen at pH 2.0 and a 68-% increase at pH 5.0 at 27°C, and a 21-% increase at pH 2.0 and a 27-% increase at pH 5.0 at 37°C.

Solubilities of ketoprofen in urea solutions were determined in order to gain an understanding of the role that solubility plays in the increased dissolution of ketoprofen in ketoprofen-urea solid dispersions. The solubility at pH 2.0 as shown in Figure 4 increases linearly at 25 and at 37°C up to approximately 1.7 moles/l (10%) urea. The stability constants (K_A) of a 1:1 complex between ketoprofen and urea [8] and the approximate energetics of the interaction at pH 2.0 are given in Table 4. The results indicate that the solution process is not spontaneous, is governed by the enthalpy which is positive and is accompanied by a positive entropy. This suggests that solubility is achieved in urea solutions primarily through a water-structure disordering occurrence. Such a mechanism has frequently been discussed by others [9, 10] and although values of K_A can be calculated for ketoprofen-urea systems it would appear that complexation plays only a minor role in the promotion of increased solubility of ketoprofen under the present conditions.

Figure 3

Dissolution of constant surface non-disintegrating pellets of 90% ketoprofen / 10% urea solid dispersions as a function of temperature and pH
○ = pH 2.0, 27°C; △ = pH 5.0, 27°C;
● = pH 2.0, 37°C; ▲ = pH 5.0, 37°C



Discussion

The solid-dispersion system of ketoprofen-urea is characteristic of a simple eutectic mixture and over the range of compositions exhibits minimal solid-solution formation, essentially occurring above 90% and below 10% ketoprofen. Thus, the most useful composition which would lead to improved dissolution of ketoprofen is the eutectic composition which occurs at 90% ketoprofen and 10% urea at an eutectic temperature of 63°C. Such a content of drug in the solid dispersion may not yield the ultrafine particles which are claimed to be produced at the eutectic composition [3] and provide increased dissolution rates [2]. A test of this eventuality is afforded by the dissolution data shown in Table 2.

Table 2

Dissolution of ketoprofen and ketoprofen-urea eutectic composition[1] in water as a function of pH and temperature

Composition	Apparent dissolution rate constant, k_{app} ($min^{-1} \cdot 10^3$)	Intrinsic dissolution rate constand, K_i ($ml/min \cdot cm^2$)
	27 °C, pH = 2.0	
Ketoprofen	6.01	0.74
Physical mixture	6.05	0.74
Solid dispersion	9.62	1.20
	27 °C, pH = 5.0	
Ketoprofen	11.01	1.35
Physical mixture	11.20	1.38
Solid dispersion	18.65	2.32
	37 °C, pH = 2.0	
Ketoprofen	13.60	1.67
Physical mixture	14.01	1.72
Solid dispersion	16.68	2.08
	37 °C, pH = 5.0	
Ketoprofen	19.27	2.37
Physical mixture	19.97	2.46
Solid dispersion	24.92	3.10

[1] 90% ketoprofen, 10% urea

The dissolution rate of a weak acid drug is influenced by several factors as described in an equation developed by *Nernst* and *Brunner* [11] and written in the form [12]

$$\frac{dQ}{dt} = \frac{DS}{h}\left[C_s\left(1+\frac{K_a}{[H^+]}\right)-C_g\right] \quad (2)$$

where Q is the amount of drug dissolved, t is time, D is the diffusion coefficient of the drug in the GI-tract fluids, S is the effective surface area of the particles, h is the thickness of the stationary layer of solvent adjacent to the particle surface, C_s is the solubility of the drug in the stationary layer (h), C_g is the concentration of drug in the bulk fluids of the GI tract and K_a and $[H^+]$ are the dissociation constant of the drug and hydrogen ion concentration, respectively. Thus, the higher the pH of the medium, the greater is C_s due to ionization of the drug and the greater the dissolution rate. Evidence of this is apparent in Table 2 for all three ketoprofen formulations.

The dissolution rate may be altered by a temperature change as well as through any one of the parameters of eqation (2). The results in Table 2 show how an increase in the temperature increases the dissolution rate. It is noted that the increase in the dissolution rate of the solid dispersion with temperature is not as great as for pure ketoprofen or its physical mixture with urea at either pH 2.0 or pH 5.0. For example the ratio of K_i of 3.10/2.32 = 1.3 for the solid dispersion whereas the corresponding ratio for the pure drug or the phyical mixture is 1.8 at pH 5.0. On the other hand, an increase in the pH of the medium increases K_i to the same degree for all three formulations. Both urea concentration and temperature increase have the effect of breaking water structure and thereby creating a more energetically favorable environment for hydrophobic solutes. An increase in solubility of ketoprofen is realized in each case as demonstrated in Figure 4. Both of these parameters affect the dissolution rate through C_s according to equation (2).

Table 3

Dissolution activation energies (kcal/mole) of ketoprofen formulations

pH	Ketoprofen	Physical mixture[1]	Solid dispersion[1]
2.0	15.0	15.6	10.2
5.0	10.4	10.7	5.4

[1] Eutectic composition: 90% ketoprofen / 10% urea
Activation energies (ΔH_a) were calculated from

$$\ln \frac{K_i(T_2)}{K_{i(T1)}} = \frac{\Delta H_a}{R}\left[\frac{T_2-T_1}{T_1T_2}\right]$$

Table 4

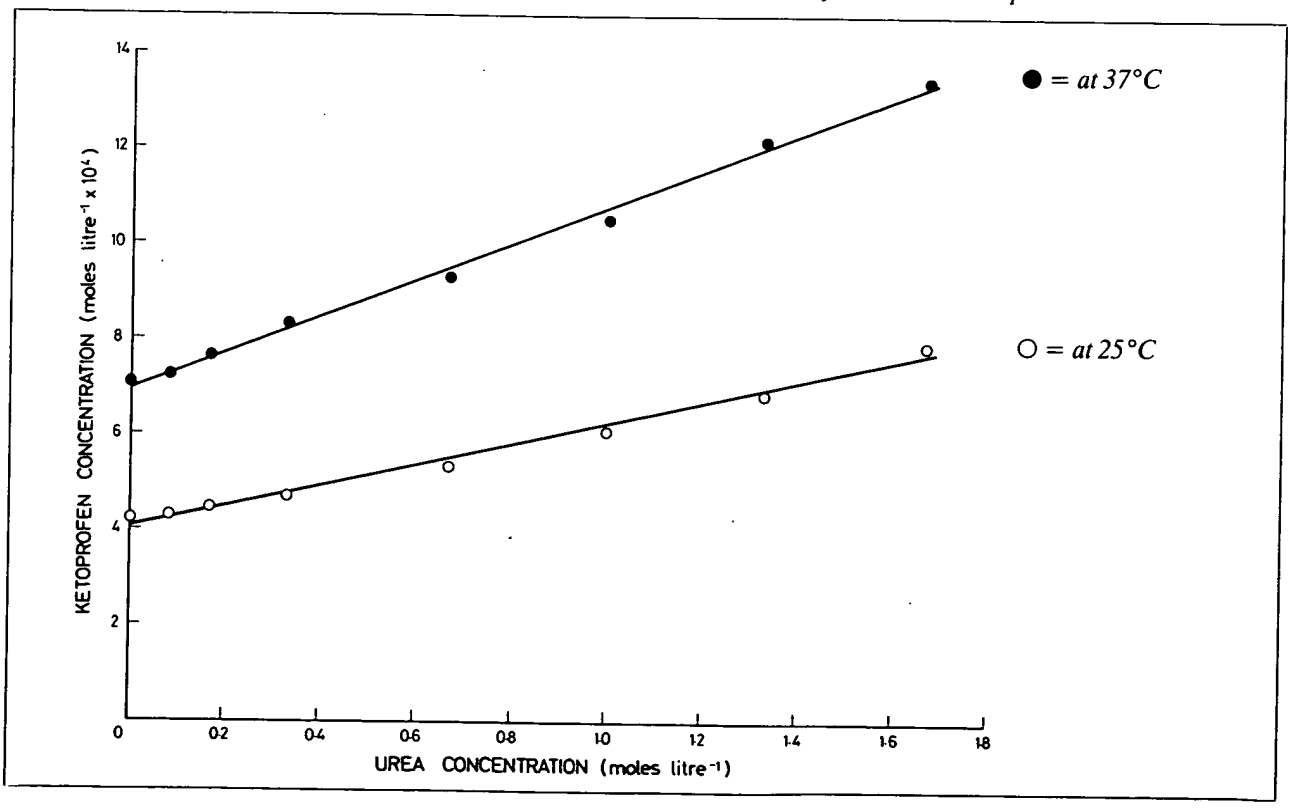
Energetics of the solution properties of ketoprofen in urea solutions at pH 2.0

Temperature (°C)	Slope	K_A[1]	ΔG (cal/mole)	ΔH (cal/mole)	$T\Delta S$ (cal/mole)
25	$2.15 \cdot 10^{-4}$	0.53	376	511	135
37	$3.82 \cdot 10^{-4}$	0.55	370	511	141

[1] 1:1 complex association constant, K_A = slope/intercept · (1-slope) (8) where slope and intercept are obtained from Figure 4. Thermodynamic quantities were determined from standard equations.

Figure 4

Solubility of ketoprofen in aqueous urea solution acidified with HCl to pH 2.0.



The fact that K_i for pure ketoprofen and its physical mixture is virtually identical indicates that an increase in C_s due to urea is negligible as far as dissolution is concerned. Extending this argument to the solid-dispersion system, the higher dissolution rates found are probably not due to a significant increase in C_s by rapidly dissolving urea from the matrix of the particles which then dissolves ketoprofen but rather due to the finer particle size according to the Kelvin equation [12],

$$C_s^{\text{micro}} = C_s \exp\left[\frac{2\gamma M}{r\varrho RT}\right] \quad (3)$$

where γ is the interfacial tension between the solid particles and the surrounding fluid, M is the molecular weight of the drug, r is the particle size, ϱ is the density of the particle, R ist the gas constant and T is the absolute temperature. As the particle size is reduced to an ultrafine state the solubility in the aqueous stationary layer increases. The increase in concentration of urea in the stationary layer is not expected to alter the γ at the solid-liquid interface although a slight increase in γ at interfaces in contact with 1–5 M urea solution has been reported [13]. It is further noted from equation (3) that an increase in temperature is predicted to diminish the influence of the fine particle size on the dissolution rate. Thus, the smaller increase in K_i with temperature as seen for the solid-dispersion formulation can be explained by the Kelvin equation.

On the basis of the above discussion it can be concluded that solid dispersions of ketoprofen-urea result in increased dissolution of ketoprofen by virtue of reduction in particle size. This should lead to improved peak plasma levels of ketoprofen after oral administration and also reduce the rest time of the tablet formulation in the GI tract. Furthermore, the high content of ketoprofen at the eutectic composition permits the tablet size to remain small, a factor considered important for patient compliance and acceptance.

Acknowledgements

The authors gratefully acknowledge the gift of ketoprofen from Poulenc Ltd., Montreal and the technical assistance of M. Poon.

References

[1] *Monkhouse D. C.* and *Lach J. L.*, J. Pharm. Sci. *61*, 1430 (1972).
[2] *Chiou W. L.* and *Riegelman S.*, J. Pharm. Sci. *60*, 1281 (1971).
[3] *Sekiguchi K.* and *Obi N.*, Chem. Pharm. Bull. *9, 866 (1961).*
[4] *Borchart H. J.* and *Daniels F.*, J. Am. Chem. Soc. *79*, 41 (1957).
[5] *Rogers J. A.* and *Anderson A. J.*, unpublished results.
[6] *El-Banna A. M.*, *Abd-El Fattah S.* and *Daabis N. A.*, Pharmazie. *29*, 396 (1974).
[7] *Dragnet-Brughmans M.*, *Azibi M.* and *Bouché R.*, J. Pharm. Belg. *34*, 267 (1979).
[8] *Connors K. A.* and *Mollica J. A.*, J. Pharm. Sci. *55*, 772 (1966).
[9] *Feldman S.* and *Gibaldi M.*, J. Pharm. Sci. *56*, 370 (1967).
[10] *Collett J. H.* and *Flood B. L.*, J. Pharm. Pharmacol. *28*, 206 (1976).
[11] *Nernst W.* and *Brunner E.*, Z. Phys. Chem. *47*, 52 (1904).
[12] *Hoener B. A.* and *Benet L. Z.*, in Modern Pharmaceutics (Banker, G. S. and Rhodes C. T., eds.) Chapt. 4 (Marcel Dekker, Inc., New York, N. Y. 1979).
[13] *Sears D. F.*, J. Colloid Interface Sci. *29*, 288 (1969).

Address of the authors

J. A. Rogers and A. J. Anderson, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

STIC-ILL 337,536 RSI.F3s

From: Berman, Alysia
Sent: Saturday, March 17, 2001 6:45 PM
To: STIC-ILL
Subject: 09/423715

Please get the following journal articles and have them delivered to my mailbox in CM1-3B19:

Aoki, S.; Okamoto, A.; Danjo, K.; Sunada, H.; Otuka, A. 'Compatibility of ibuprofen and ethenzamide' Drug Dev. Ind. Pharm. (1997) 23(6):561-565.

Murtha, John L.; Ando, HOward Y. 'Synthesis of hte cholesteryl ester prodrugs chloesteryl ibuprofen and cholesteryl flufenamate and their formulation into phospholipid microemulsions' J. Pharm. Sci. (1994) 83(9):1222-8.

Goroshevich, R. V.; Kosmynin, A. S.; Rozhanskaya, A. E.; Tkachenko, M. L. 'Physicochemical study of drug binary systems' Khim.-Farm. Zh. (1992) 26(2):73-6.

Mura, P.; Liguori, A.; Bramanti, G. 'Solid dispersions of ibuprofen in urea. Effects of urea on dissolution' Farmaco, Ed. Prat. (1986) 41(12):377-87.

Rogers, J. A.; Anderson, A. J. 'Physical characteristics and dissolution profiles of ketoprofen-urea solid dispersions' Pharm. Acta Helv. (1982) 57(10-11):276-81.

Thank you.

Alysia Berman
Patent Examiner
CM1-3D06
(703) 308-4638
1619

COMPLETED

? d s2/3/all

Display 2/3/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05638095 BIOSIS NO.: 000083111240
SOLID DISPERSIONS OF IBUPROFEN IN UREA EFFECTS OF UREA ON DISSOLUTION
AUTHOR: **MURA P**; LIGUORI A; BRAMANTI G
AUTHOR ADDRESS: DIPARTIMENTO DI SCIENZE FARMACEUTICHE-UNIV. DI FIRENZE, ITALY.
JOURNAL: FARM ED PRAT 41 (12). 1986 (RECD. 1987). 377-387. **1986**
FULL JOURNAL NAME: Farmaco Edizione Pratica
CODEN: FRPPA

IL FARMACO
Anno XLI - N. 12 EDIZIONE PRATICA Dicembre 1986

SOLID DISPERSIONS OF IBUPROFEN IN UREA. EFFECTS OF UREA ON DISSOLUTION

P. MURA - A. LIGUORI - G. BRAMANTI

DIPARTIMENTO DI SCIENZE FARMACEUTICHE - UNIVERSITÀ DI FIRENZE (ITALY)

SUMMARY. — *Solid dispersions of Ibuprofen in urea were prepared by the solvent method. These dispersions were characterized using differential thermal analysis and X-ray diffraction. The system proved to be a simple eutectic mixture with eutectic composition of 90% Ibuprofen and 10% urea. In comparison with the drug alone, the physical mixtures and even more the solid dispersions showed a marked increase in the dissolution rate. The importance of the solubilizing effect of urea in the enhancement of drug dissolution was also evaluated.*

RIASSUNTO. — *Dispersioni solide di Ibuprofen in urea, ottenute col metodo del solvente, sono state caratterizzate tramite analisi termica differenziale e analisi di diffrazione a raggi X. Il sistema Ibuprofen-urea è risultato essere una semplice miscela eutettica, il cui punto eutettico è al 90% di Ibuprofen e 10% di urea. È stato osservato un marcato aumento della velocità di dissoluzione delle mescolanze fisiche ed ancor più delle dispersioni solide rispetto al farmaco da solo. È stata anche valutata l'importanza dell'effetto solubilizzante dell'urea nell'aumento di dissoluzione dell'Ibuprofen.*

Introduction

Ibuprofen [2-(4-isobutylphenyl)propionic acid] is an analgesic with minor antiinflammatory properties, employed as a substitute of the steroids. Its very slight solubility in water and its low dissolution rate may lead to incomplete and erratic gastrointestinal absorption (1).

It is well established that dissolution is frequently the rate-limiting step in the gastrointestinal absorption of a drug from a solid dosage form.

Since the dissolution rate is directly proportional to the surface area exposed to dissolution, it is possible to increase this rate by decreasing the particle size of the drug.

Among the various approaches to achieve particle size reduction, the use of solid dispersions in a water soluble carrier, first proposed by Sekiguchi and Obi (2), has often proved to be very successful (3).

The enhancement of drug dissolution rate by solid dispersions has been attributed to the state of fine subdivision of crystalline particles throughout the carrier.

Since there are several reports on the solubilizing effects of carriers (such as urea), also the study of the solubility into solution of carriers appeared of some interest in order to evaluate the role of solubilization in the enhancement of drug dissolution rate.

The aim of the present work is to investigate the possibility of improving the dissolution rate of Ibuprofen via solid dispersions techniques, using urea as a carrier, and to evaluate the role of solubilization in the enhancement of Ibuprofen dissolution by urea.

Experimental

Materials

Ibuprofen (Sigma Chemical Co.) and urea (Merck) were used as obtained from the supplier. Methanol was analytical grade.

Preparation of solid dispersions

Solid dispersions were prepared by the solvent method. Coprecipitates of Ibuprofen and urea in different ratios were prepared by dissolving the components in the minimum amount of methanol followed by evaporation of the solvent *in vacuo* at room temperature. The residue was finely ground, sieved to a particle size range of 75-150 μm and stored in a desiccator.

Preparation of physical mixtures

Physical mixtures were prepared by simple mixing of the two ingredients possessing the same particle size range (75-150 μm), in various proportions. Pure crystalline Ibuprofen (75-150 μm) served as control sample.

Differential thermal analysis

The differential thermal analyzer (Mettler TA 2000) was calibrated using indium. All samples were run from 27 to 150° at a scanning rate of 4°/min. Nitrogen was used as purge gas at 18 ml/min. Sensitivity was 200 μV/f.s., chart speed was 1 cm/min.

X-ray diffraction

All X-ray diffraction spectra (Philips PW 1130 diffractometer) were obtained by scanning at 2°/min, in terms of a 2 θ angle.

ticle size reduction,
:r, first proposed by
: very successful (3).
olid dispersions has
crystalline particles

ıg effects of carriers
solution of carriers
ole of solubilization

e the possibility of
l dispersions techni-
ole of solubilization

were used as obtained

:thod. Coprecipitates
ed by dissolving the
'ed by evaporation of
.e was finely ground,
ɔred in a desiccator.

ɔf the two ingredients
various proportions.
sample.

was calibrated using
nning rate of 4°/min.
/ity was 200 μV/f.s.,

diffractometer) were

Dissolution rate studies

The dissolution rate of Ibuprofen from solid dispersions and physical mixtures with urea was determined at 37±0.1°.

The dissolution apparatus was similar to that employed by Levy and Hayes (4). A 400 ml beaker containing 300 ml of distilled water, maintained at 37±0.1° and agitated at 100 r.p.m. by a glass stirrer blade connected to a constant-speed stirring motor, was used. At suitable time intervals a 3.0 ml sample was withdrawn, filtered by means of a filter-syringe (pore size 0.45 μm) and determined by second derivative ultraviolet spectrometry at 264 nm.

This technique is particularly useful in pharmaceutical analysis to assay drugs with low absorptivity values (5).

Spectral measurements were made with a Perkin Elmer Mod. 552S spectrometer. The second derivative spectra were recorded using the following instrumental parameters: scan speed 120 nm/min; chart speed 60 mm/min; response time 0.5 sec; recorder range ±0.05.

Solubility studies

Excess drug was added to 30 ml of water or aqueous solutions of different urea concentrations. The solutions in stoppered glass tubes were continuously shaked in a water bath at 20±0.1° and 37±0.1° for 24 hours. Samples were withdrawn and filtered through a millipore filter (pore size 0.45 μm) and then submitted to 264 nm second derivative spectrophotometry.

Results and discussion

Differential termal analysis

The D.T.A. thermograms of Ibuprofen-urea solid dispersions and pure Ibuprofen are reported in Fig. 1.

The thermograms are characterized by two endothermic peaks over the range 10-80% Ibuprofen, corresponding to the eutectic composition and to the urea melting peak respectively. The endotherms for urea gradually shift to lower temperatures as the urea content of the solid dispersion diminishes and at 10% urea, the corresponding endotherm vanishes. At 98% Ibuprofen the endothermic melting peak of Ibuprofen appears.

Phase diagram

The phase diagram for Ibuprofen-urea compositions, constructed by means of the DTA data, is shown in Fig. 2 and is typical of a simple eutectic mixture.

The eutectic composition was found to be 90% Ibuprofen and 10% urea.

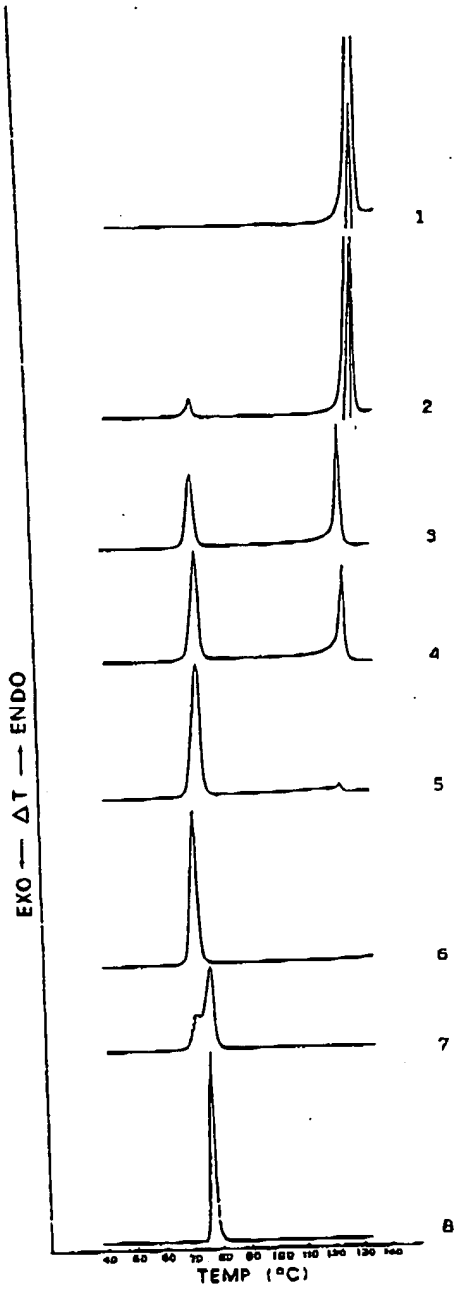


Fig. 1

DTA thermograms of Ibuprofen-urea system.

1) 100% Urea; 2) 10% Ibuprofen; 3) 50% Ibuprofen; 4) 60% Ibuprofen; 5) 80% Ibuprofen; 6) 90% Ibuprofen; 7) 98% Ibuprofen; 8) 100% Ibuprofen.

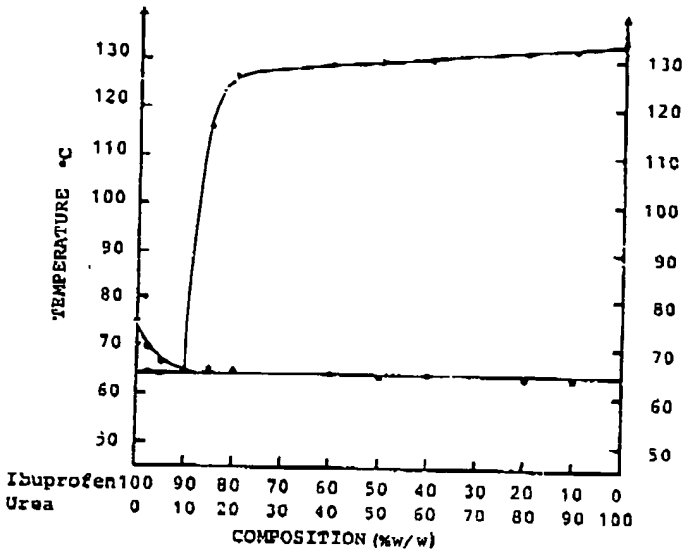


Fig. 2

Phase diagram of the binary system Ibuprofen-urea.

X-ray diffraction

The X-ray diffraction spectra of the pure urea and pure Ibuprofen, and spectra of the solid dispersion and physical mixture of 10% Ibuprofen are shown in Fig. 5.

Spectra indicate that the Ibuprofen and urea in the solid dispersion have same crystalline form as that of the pure compounds.

X-ray diffraction data also show that the Ibuprofen-urea system should be described as a simple eutectic mixture, as found by DTA studies.

The presence of the typical X-ray diffraction peaks of Ibuprofen in the solid dispersion clearly indicates that the sample is not a solid solution but an eutectic mixture.

The height of these peaks, which are comparable with those obtained from the physical mixture, also indicates the negligibility of solid-solid solubility.

Interaction in water

Equilibrium solubility experiments were conducted at two temperatures to determine the extent of interaction between Ibuprofen and urea in aqueous solutions. Fig. 4 shows that urea interacts with Ibuprofen: this interaction is evident from the linear increase in the solubility of Ibuprofen when urea concentration increases.

The straight lines were obtained by the method of least squares; the linear correlation coefficient was 0.9978 at 20° ($P<0.001$) and 0.9974 at 37° ($P<0.001$).

em.

4) 60% Ibuprofen;
8) 100% Ibuprofen.

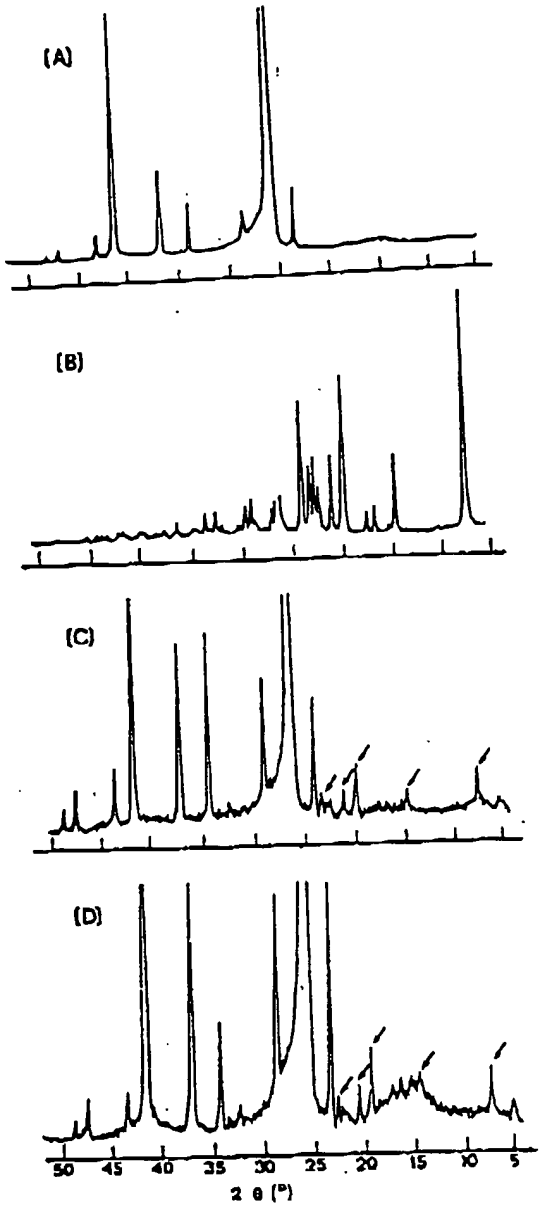


Fig. 3

X-ray diffraction spectra of: (A) pure urea, (B) pure Ibuprofen, (C) solid dispersion of 10% Ibuprofen and (D) physical mixture of 10% Ibuprofen. Arrows indicate diffraction peaks due to the presence of Ibuprofen cristallites.

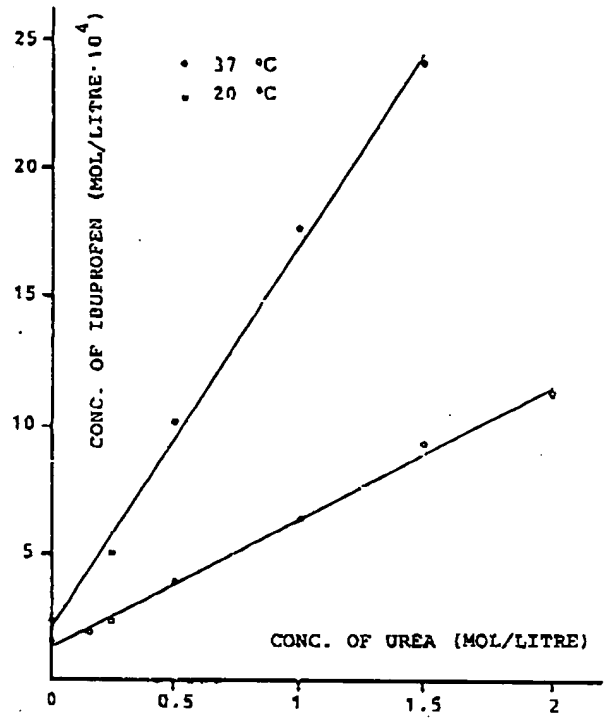


Fig. 4

Solubility of Ibuprofen in aqueous urea solutions.

The stability constant (K) of a 1:1 complex between Ibuprofen and urea and the approximate energetics of the interaction are given in Table I.

TABLE I

Thermodynamic parameters of Ibuprofen-urea system.

Temperature °C	K	ΔG kJ/mole	ΔH KJ/mole	ΔS J/mole · grado
20	3.96	—5.355	26.354	101.38
37	7.17	—5.075	26.354	101.38

The constant K = slope/intercept · (1-slope) (6), was obtained from equations data of the straight lines of Fig. 4. The thermodynamic quantities were determined from standard equations.

(C) solid dispersion n. Arrows indicate ristallites.

The ΔG and ΔS values, respectively negative and positive, indicate a spontaneous process. Similar negative ΔG values were reported by other authors (7-9) who have solubilized non-polar solutes in urea solutions. The positive entropy and enthalpy changes are in accordance with the theory that the enhancement of dissolution of hydrophobic compounds in the aqueous urea solutions is accomplished by breaking of the water structure by urea (9, 10).

The increase in entropy obtained by this "disruption" makes the association of nonpolar molecules by hydrophobic bonding thermodynamically less favorable.

The increase of both urea concentration and temperature results in the increase of such breaking of water structure and creates an energetically more favorable environment for hydrophobic solutes.

Nevertheless the K values for the Ibuprofen-urea system show that also complexation plays a role in the promotion of increased solubility of Ibuprofen under the present conditions.

Dissolution rate studies

Fig. 5 and Table II summarize the enhanced dissolution rates obtained from the Ibuprofen-urea physical mixtures and coprecipitates.

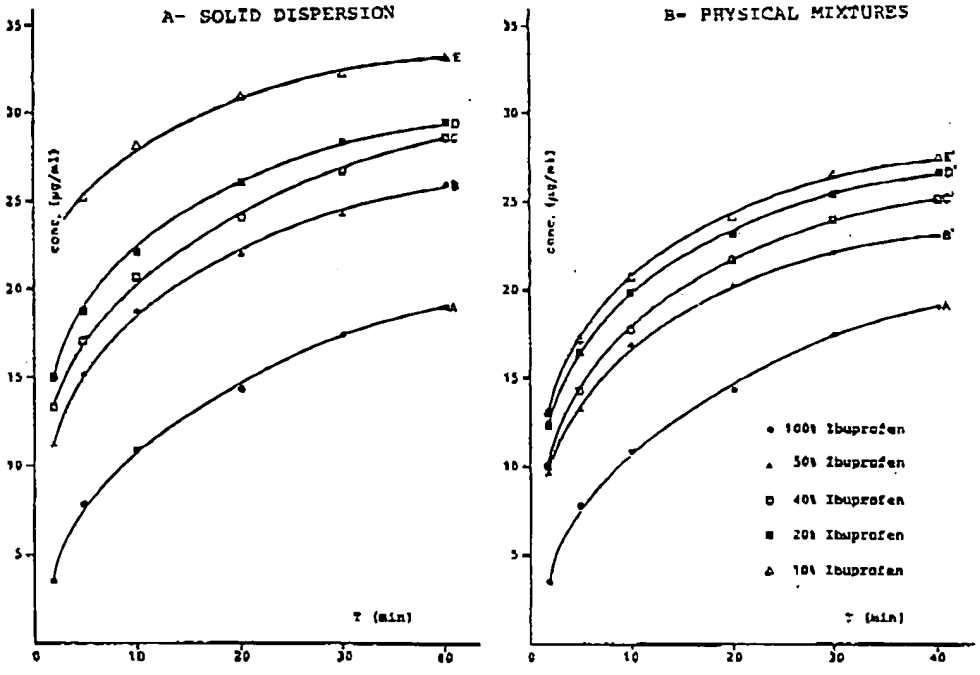


Fig. 5

Dissolution rates of Ibuprofen-urea solid dispersions (A) and corresponding physical mixtures (B).

TABLE II

Dissolution studies of Ibuprofen from solid dispersions and physical mixtures with urea.

Samples	Amounts dissolved (μg/ml)			Relative dissolution rate		
	2 min	5 min	40 min	2 min	5 min	40 min
A -Pure drug	3.50	7.80	19.00	1.00	1.00	1.00
B -Solid dispersion 50% Ibuprofen	11.25	15.26	26.00	3.21	1.90	1.37
B'-Physical mixture 50% Ibuprofen	9.73	13.49	23.10	2.78	1.73	1.22
C -Solid dispersion 40% Ibuprofen	15.40	17.10	28.60	3.83	2.19	1.50
C'-Physical mixture 40% Ibuprofen	10.04	14.44	25.10	2.86	1.85	1.33
D -Solid dispersion 20% Ibuprofen	15.00	18.82	29.45	4.28	2.41	1.55
D'-Physical mixture 20% Ibuprofen	12.30	16.65	26.70	3.52	2.13	1.40
E -Solid dispersion 10% Ibuprofen	23.17	25.20	33.20	6.62	3.23	1.75
E'-Physical mixture 10% Ibuprofen	13.15	17.00	27.50	3.76	2.18	1.45

Relative dissolution rate data were calculated by evaluating the amount of Ibuprofen dissolved from various preparations at 2.5, and 40 min, and dividing it by the amount of the drug dissolved from the pure crystalline sample at the same time intervals.

'r. - vol. 41 - fasc. 12

ɔsitive, indicate a
reported by other
ɛa solutions. The
; with the theory
ɔmpounds in the
ıe water structure

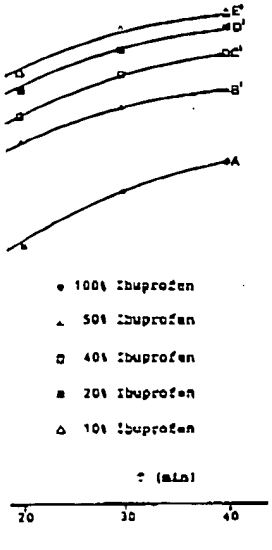
ɔtion" makes the
ding thermodyna-

ture results in the
s an energetically

ɛm show that also
bility of Ibuprofen

ion rates obtained
ates.

HYSICAL MIXTURES



responding physical

The coprecipitate at the eutectic composition (not shown), displayed the same dissolution characteristics as the pure Ibuprofen.

Significant increase in dissolution for solid dispersions and physical mixtures has been found only when the per cent of urea was equal to or higher than, 50% w/v.

The solid dispersions were found to dissolve slightly faster than their respective physical mixtures.

This suggests that the enhancement of Ibuprofen dissolution is achieved primarily through a solubilization effect by the carrier in the microenvironment immediately surrounding the drug particle in the early stage of dissolution; that is urea increases the solubility of Ibuprofen in the diffusion layer, thereby increasing its dissolution rate.

The significance of solid dispersions formation can be shown by comparing the relative dissolution rates obtained for various samples at short times.

In particular the sample "E" (solid dispersion containing 10% of the drug) at 2 min dissolved 6.62 times faster than the crystalline Ibuprofen. It is not possible to attribute such an increase in the dissolution rate to a local effect, since the physical mixture of identical composition (sample "E'") dissolved at the same time only 3.76 times faster than the pure drug.

It has to be concluded that such difference may be attributed to better wettability and dispersibility and to the drug particle size reduction taking place in the solid dispersion when a large excess of urea is used.

Conclusions

The present investigation has shown that the addition of urea to Ibuprofen both in the form of physical mixture and even more in the form of solid dispersion, can markedly enhance the *in vitro* dissolution of Ibuprofen.

The higher the urea quantity, the greater the effect evidenced.

Besides, from the results of solubility studies it may be concluded that the urea solubilizing effect is an important factor regulating the dissolution rate of Ibuprofen from Ibuprofen-urea mixtures.

The authors feel indebted to Prof. G.G.T. Guarini (*Dept. of Chemistry*) *for valuable suggestions concerning the recording and interpretation of thermal curves. The authors are also grateful to* Dr. L. Poggi (*Inst. of Mineralogy*) *for help in performing and explaining the X-ray diffraction spectra, and to* Mr. Luca Ceccarelli *for his technical assistance.*

REFERENCES

1) GIBALDI M., Introduction to Biopharmaceutics, Lea and Febiger, p. 21; 1971.
2) SEKICUCHI K., OBI N., *Chem. Pharm. Bull.*, 9, 866; 1961.
3) CHIOU A.L., RIEGELMAN S., *J. Pharm. Sci.*, 60, 1281; 1971.

4) LEVY
5) MAHP
Chem.
6) CONN
7) WETI
508;
8) NOZA
9) FELDI
10) COLLI

Perve

own), displayed

s and physical
vas equal to or

aster than their

tion is achieved
the microenvi-
early stage of
in the diffusion

be shown by
ous samples at

ng 10% of the
: Ibuprofen. It
ution rate to a
n (sample "E'")
re drug.
ibuted to better
eduction taking
used.

on of urea to
n more in the
tro dissolution

:videnced.
be concluded
regulating the
ures.

: *of Chemistry*)
ution of thermal
of Mineralogy)
spectra, and to

ger, p. 21; 1971.

4) LEVY G., HAYES B.A., *New Eng. J. Med.*, **262**, 1053; 1960.
5) MAHROUS M.S., ABDEL-KHALEK M.M., ABDEL-HAMID M.E., *J. Assoc. Off. Anal. Chem.*, **68**, 535; 1985.
6) CONNORS K.A., MOLLICA J.A., *J. Pharm. Sci.*, **55**, 772; 1966.
7) WETLAUFER D.B., MALIK S.K., STOLLER L., COFFIN R.L., *J. Am. Chem. Soc.*, **86**, 508; 1964.
8) NOZAKI Y., TANFORD C., *J. Biol. Chem.*, **238**, 4074; 1963.
9) FELDMAN S., GIBALDI M., *J. Pharm. Sci.*, **56**, 370; 1967.
10) COLLETT J.H., FLOOD B.L., *J. Pharm. Pharmac.*, **28**, 206; 1976.

Pervenuto in Redazione il 29 *Luglio* 1986.